STUDY 3 OF 3

UNIVERSITY of ROCHESTER MEDICAL CENTER

EASTMAN DENTAL CENTER
SCHOOL OF MEDICINE AND DENTISTRY
SCHOOL OF NURSING
STRONG MEMORIAL HOSPITAL
UNIVERSITY MEDICAL FACULTY GROUP

DEPARTMENT OF MEDICINE
PULMONARY AND CRITICAL CARE UNIT

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Consent Form

Study Title: Ultrafine Particle Effects on Dendritic Cells in Asthma ("ASTHMACON")

Principal Investigator: Mark W. Frampton, MD

Introduction:

This consent form describes a research study and what you may expect if you decide to participate. You are encouraged to read this consent form carefully and to ask the person who presents it any further questions you may have before making your decision whether or not to participate. This study is being conducted by Dr. Mark Frampton of the Pulmonary and Critical Care Medicine Division of the Department of Medicine at the University of Rochester Medical Center.

You are being asked to participate in this study because you are a nonsmoker, 18 to 50 years of age, with asthma.

Purpose of Study

The purpose of this research study is to determine if people exposed to very small ("ultrafine") particles normally present in the outdoor air develop temporary changes in the blood cells that control the body's immune responses. We are also testing whether people with a specific kind of genetic makeup are more susceptible to effects on these cells. The levels of pollutants to which you will be exposed will not be higher than what you could be exposed to if you visited many major cities around the world.

Description of Study Procedures

If you agree to participate in this study, you will be asked to come to the Clinical Research Center (CRC) or the Pulmonary and Critical Care Unit on 5 separate days, including 2 overnight stays, for a total of about 63 hours over approximately 6 weeks.

On the first day (**Visit 1**), you will complete a standardized questionnaire for assessment of respiratory symptoms and medical history. You will have a medical and physical examination, routine breathing tests (spirometry), an electrocardiogram (ECG), and a blood test, including genetic testing. The amount of blood to be drawn will be 1-2 tablespoons. Response to breathing a methacholine aerosol will be assessed by spirometry. Methacholine is a standard drug used to test for asthma. These procedures are described in detail below. A pregnancy test will be performed in female subjects. The pregnancy test must be negative. Visit 1 takes approximately 3 hours and will determine whether you are eligible to participate in this study.

You must be able to avoid the medications listed below, for 1 week before starting the study, until the study is finished:

- Non-steroidal anti-inflammatory drugs such as ibuprofen, naproxen, including aspirin
- Prednisone
- Vitamins C and E
- Antihistamines
- Anti-oxidants
- Fish oil
- Niacin
- Arginine
- Over-the-counter decongestants

does not discuss use of prescription meds

You will also be asked to avoid caffeine throughout the study, starting with dinner the evening before visit 2.

One or more days after Visit 1, you will be asked to come to the CRC at 11:30 AM (**Visit 2**). You will be rescheduled if you have experienced an upper or lower respiratory tract illness within the past 6 weeks, or any other acute illness within the past week. Women will be asked about their latest menstruation and a pregnancy test will be conducted. If you are pregnant your participation in the study will end and you will receive full compensation for the exposure day. You will be given lunch. At about 12:30 PM you will have the following procedures: blood pressure, heart rate, attachment of a finger clip and recorder for the measurement of oxygen in the blood (oximetry), completion of a questionnaire about symptoms, measurement of the amount of nitric oxide in your exhaled breath, removal of blood (5-6 tablespoons) from a vein in your arm, collection of a sample of your urine, exhaled breath condensate, and spirometry. These procedures will take about 3 hours. You will be given dinner that evening, and will stay on the CRC overnight.

The next morning you will have a light breakfast at 6:15 AM. At 7:00 AM you will be transported by wheelchair to the Kornberg Medical Research Building, where you will have a 2-

hour exposure to either clean air, or clean air containing concentrated outdoor ultrafine particles. You will not be told which exposure you are receiving, and the investigators will not know. Only the person operating the exposure equipment will know which exposure is being given. The order of giving air or particles will be chosen at random (like flipping a coin).

The exposure will be done inside a Plexiglas chamber (6 x 5 x 3.5 feet, 98 cubic feet) in a research building at the Medical Center. The chamber will be under negative air pressure, which may make your ears pop, like going up in an elevator. On the particle exposure day, the air you breathe will contain particles from outside the building that have been concentrated about 10 to 20 times more than their concentration outdoors. The amount of particles you will be exposed to will depend on the amount of pollution in the outside air on the day of your exposure. A trained investigator will be nearby to observe you at all times. A physician will be on call in the facility during the entire exposure session.

It is not expected that the exposures in this study will cause any symptoms. If it appears you are experiencing any problems, or you develop any symptoms of discomfort, the exposure will be stopped immediately. In addition, you may choose to stop the exposure at any time for any reason. If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and for any further payments.

After the exposure, you will be transported back to the CRC, where blood pressure, heart rate, blood oxygen saturation, and spirometry will be measured, and you will be given a questionnaire to record symptoms. You will be given lunch at 11:30. At 12:30 PM the following measurements will be made: blood pressure, heart rate, oximetry, a symptom questionnaire, blood tests (2-3 tablespoons), urine collection, and spirometry. All of these measurements are described in detail below. You will then go home. The total time for Visit 2 will be about 27 hours, including the overnight stay.

You will return the next morning (**Visit 3**) at 8:00 AM, approximately 24 hours after exposure. Upon arriving for Visit 3, all of the measurements listed above will be repeated. The amount of blood drawn will be 5-6 tablespoons. Visit 3 will take approximately 3 hours.

At least 3 weeks after Visit 2, you will return for **Visits 4 and 5**. The procedures performed on Visits 2 and 3 will be repeated. You will have completed the study after Visit 5.

The measurement procedures are described below:

1) Routine breathing tests (spirometry). This test requires you to perform 3 to 5 forceful exhalations after a deep breath. This test is performed routinely on patients and does not carry significant risks.

2) Methacholine challenge. This test involves inhaling methacholine. It causes constriction of the airways or breathing tubes. The amount of constriction of the breathing tubes is determined with spirometry, a breathing test. People with asthma respond to lower amounts of methacholine than people without asthma. You will inhale 5 breaths of methacholine in gradually increasing concentrations, and do a breathing test 30 seconds after inhaling each

concentration. The test will be stopped when the breathing test decreases 20%, or when you have reached the highest amount of methacholine. You will then inhale albuterol; this opens up the breathing tubes. Albuterol is often used in the treatment of asthma. The breathing test will then be repeated one more time. The methacholine challenge test will be done only if your breathing test is normal to begin with. If the breathing test is not normal, you will be asked to inhale the albuterol only, and the breathing test will be repeated.

3) Blood drawing. Blood will be removed from a vein in your arm for the study of blood cells and fluids. The amount of blood taken at each blood drawing will be no more than 5-6 tablespoons (80 ml) at a time, no more than 200 ml over the 2 visits of an exposure session, and no more than 420 ml over the whole study. In this study, blood samples will be stored for possible additional future research. These samples will be labeled with a code, not your name.

4) Measurement of nitric oxide in the exhaled air. Nitric oxide is a gas that is produced and released in very small quantities by many cells in the body. It is released by cells of the respiratory tract, and can be measured in air exhaled from the lungs. People who have irritation or inflammation in the lungs sometimes have increased amounts of nitric oxide in their exhaled air. We will determine whether exposure to ultrafine particles causes exhaled nitric oxide levels to increase. You will be asked to perform a series of breathing maneuvers through a mouthpiece. You will be asked to hold your breath for 10 seconds and to breathe out against a slight resistance. This will be done 6 times, each time breathing out at different speeds. During these tests, you will be inhaling air that has been purified to remove nitric oxide.

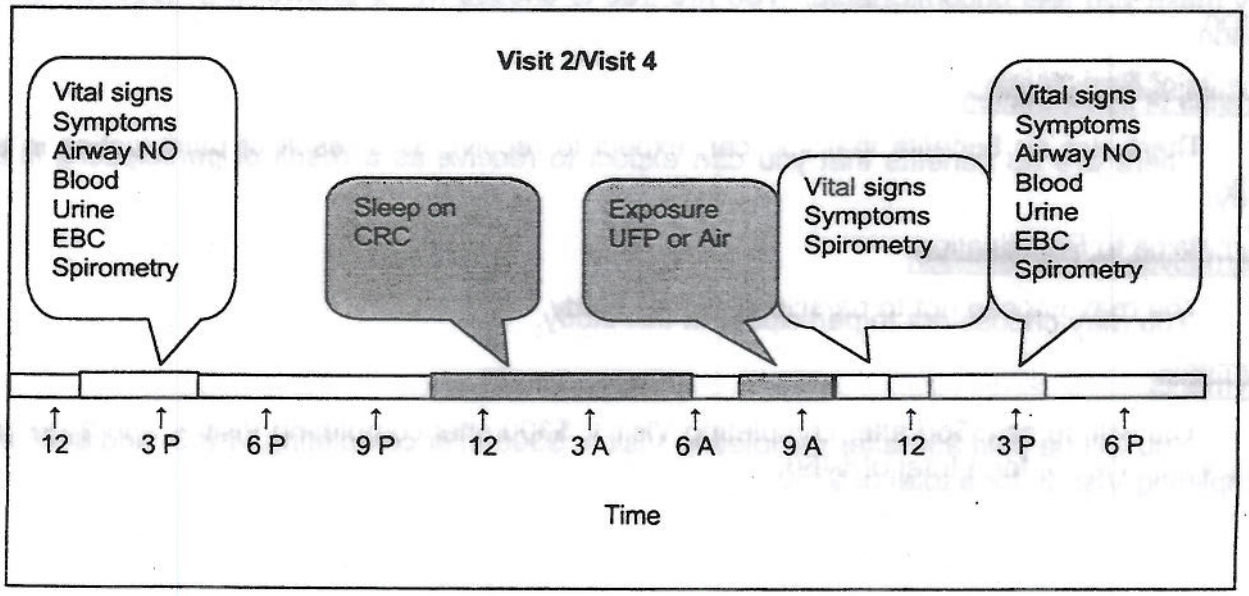
You will also be asked to repeat these breathing tests while inhaling a low concentration (up to 10 parts per million) of nitric oxide from a bag and then hold your breath for 3 to 5 seconds followed by a slow exhalation. This will be repeated 2 times. This concentration of nitric oxide is higher than that found in fresh air, but is below the levels that have been measured in air from the stomach of some people, and is below the levels of nitric oxide that have been safely used to treat various kinds of lung disease. There are no known undesirable effects from breathing this concentration of nitric oxide.

5) Exhaled breath condensate (EBC). You will be asked to breathe in and out of a small tube for 15 minutes while wearing a nose clip in order to collect the water droplets form your airways.

The table below shows when these procedures will be performed.

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
History and Physical Examination	X				
Blood Pressure and Heart Rate	X	X	X	X	X
Oximetry	X	X	X	X	X
Blood Drawing	X	X	X	X	X
Urine Sample		X	X	X	X
Pregnancy Test	X	X		X	
Electrocardiogram	X				
Methacholine challenge	X				
Air/Particle Exposure		X		X	
Exhaled breath condensate		X	X	X	X
Symptom Questionnaire		X	X	X	X
Spirometry	X	X	X	X	X
Measurement of Exhaled Nitric Oxide		X	X	X	X

The Figure below shows what happens on Visits 2 and 4, when you stay overnight and have the exposure the next day.



Number of Subjects

10 subjects are needed to complete this study.

Risks of Participation

The particles you breathe will come from outdoor air pollution that we all breathe. The number of particles will be higher than normally occur outdoors in Rochester.

These low concentrations of **particles** are not expected to cause any symptoms. Large epidemiology studies indicate that exposure to air pollution particles may increase the risk of having a heart attack or other heart problems in people who have heart disease. If you have significant symptoms such as chest pain or shortness of breath, the exposure will be stopped.

Methacholine challenge is a safe test that is often done in people with asthma. It can rarely cause coughing or shortness of breath, similar to a mild asthma attack. The effects of methacholine are reversed by inhaling albuterol.

Breathing tests (spirometry) may induce lightheadedness as a result of taking a deep breath 3 times in a row.

Blood drawing may cause pain and bruising at the place where the blood is taken. Rare complications from blood drawing include, but are not limited to, blood clots, infection, and light-headedness or fainting.

Measuring the **exhaled nitric oxide** may cause slight discomfort and drying of the nose or mouth.

Collection of **exhaled breath condensate** does not carry any risks.

Questions on the health questionnaire will ask about smoking and drinking habits, which may make you feel uncomfortable. You are free to choose not to answer any question for any reason.

Benefits of Participation

There are no benefits that you can expect to receive as a result of participating in this study.

Alternatives to Participation

You may choose not to participate in this study.

Payments

You will be paid $50 after completing Visit 1, $300 after completing Visit 3, and $400 after completing Visit 5, for a total of $750.

Sponsor Support

The University of Rochester is receiving payment from the Department of Health and Human Services and the U.S. Environmental Protection Agency for conducting this research study.

Confidentiality of Records and HIPAA Authorization

While we will make every effort to keep information we learn about you private, this cannot be guaranteed. Other people may need to see the information. While they normally protect the privacy of the information, they may not be required to do so by law. Results of the research may be presented at meetings or in publications, but your name will not be used.

The Federal Health Insurance Portability and Accountability Act (HIPAA) requires us to get your permission to use health information about you that we either create or use as part of the research. This permission is called an Authorization. We will use your health information to conduct the study, to monitor your health status, to measure the effects of inhalation of particles, and to determine research results. Health information is used to report results of research to sponsors and federal regulators. It may be audited to make sure we are following regulations, policies, and study plans. Strong Health policies let you see and copy health information after the study ends, but not until the study is completed. If you have never received a copy of the Strong Health HIPAA Notice, please ask the investigator for one.

To meet regulations or for reasons related to this research, the study investigator may share a copy of this consent form and records that identify you with the following people: the University of Rochester; the Department of Health and Human Services; National Institute of Environmental Health Sciences and the U.S. Environmental Protection Agency.

If you decide to take part, your Authorization for this study will not expire unless you cancel (revoke) it. The information collected during your participation will be kept indefinitely. You can always cancel this Authorization by writing to the study investigator. If you cancel your Authorization, you will also be removed from the study. However, standard medical care and any other benefits to which you are otherwise entitled will not be affected. Canceling your Authorization only affects uses and sharing of information after the study investigator gets your written request. Information gathered before then may need to be used and given to others.

As stated in the section on Voluntary Participation below, you can also refuse to sign this consent/Authorization and not be part of the study. You can also tell us you want to leave the study at any time without canceling the Authorization. By signing this consent form, you give us permission to use and/or share your health information as stated above.

Contact Persons

If you have any questions regarding this research, or any concerns or complaints, or if you believe that you have suffered from a research-related injury, you should contact Dr. Mark Frampton at (585) 275-4161.

If you have any questions about your rights as a research subject, you may contact the Human Subjects Protection Specialist at the University of Rochester Research Subjects Review Board, Box 315, 601 Elmwood Avenue, Rochester, NY 14642-8315, telephone (585) 276-0005; for long-distance you may call toll-free, (877) 449-4441. You may also call this number if you cannot reach the research staff or wish to talk to someone else.

Voluntary Participation

Participation in this study is voluntary. You are free not to participate or to withdraw at any time, for whatever reason, without risking loss of present or future care you would otherwise expect to receive. In the event that you do withdraw from this study, the information you have already provided will be kept in a confidential manner.

Signature/Dates

Study Subject:

I have read the contents of this consent form, and have been encouraged to ask questions. I have received answers to my questions. I agree to participate in this study. I have received (or will receive) a signed copy of this form for my records and future reference. I agree to have my blood samples used for other research in the future. Samples will be identified only with my initials and a number, not my name.

_____**PRINT NAME**

_____**SIGNATURE** _____**DATE**

Person Obtaining Consent:

I have read this form to the subject and/or the subject has read this form. The subject will receive a signed copy of this consent form. An explanation of the research was given and questions from the subject were solicited and answered to the subject's satisfaction. In my judgment, the subject has demonstrated comprehension of the information.

_____**PRINT NAME AND TITLE**

_____**SIGNATURE** _____**DATE**

PROTOCOL

Exposure to Concentrated Outdoor Ultrafine Particles in Healthy Subjects (UPCON)

April 5, 2006

Investigators:

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I. HYPOTHESES

Exposure to ambient air particulate matter (PM) is associated with increased risk for cardiovascular mortality, cardiac arrhythmias, and myocardial infarction, but the mechanisms have not been defined. There is an increased risk for respiratory and cardiac problems, especially among the elderly with underlying respiratory or cardiovascular disease.

Increasing evidence indicates that exposure to PM affects endothelial function. The vascular endothelium plays a key regulatory role in assuring tissue perfusion, maintaining vascular integrity, responding to injury, initiating inflammation, and limiting coagulation. A dysfunctional vascular endothelium is the earliest manifestation of atherosclerotic vascular disease, and the mechanisms involve signaling mediated by reactive oxygen species (ROS) and nitrogen species. Endothelial dysfunction may result in a number of vascular changes, such as impaired vasodilation, development of prothrombotic and pro-inflammatory states, and proliferation in the arterial wall, all of which contribute to the formation and progression of chronic atherosclerotic lesions (Widlansky et al. 2003). An increased incidence of adverse cardiovascular events has been reported in patients with impaired endothelial function compared

to patients with preserved endothelial function (Gokce et al. 2002; Gokce et al. 2003; Heitzer et al. 2001; Neunteufl et al. 2000; Perticone et al. 2001).

Ultrafine particles (UFP, <100 nm diameter) may be particularly important with regard to cardiovascular effects because of their potential to evade clearance mechanisms and enter the lung interstitium and vascular space. UFP at the same mass concentration in the air have a much higher number concentration and surface area than larger particles. For example, in order to achieve a low airborne concentration of 10 $\mu g/m^3$, 2.4×10^6 of ultrafine 20-nm particles/cm^3 are needed; in contrast, only 1 particle/cm^3 of 2.5-μm particles is needed to reach the same concentration (Oberdörster et al. 1995). UFP are biologically more reactive than larger-sized particles, and appear to elicit effects in animals at lower concentrations. Furthermore, UFP have a high specific surface area and carry an increased burden of ROS. We have demonstrated in healthy nonsmokers that inhalation of laboratory-generated carbon UFP reduces the pulmonary diffusing capacity for carbon monoxide, reduces blood leukocyte expression of adhesion molecules, and blunts the post-exercise increase in flow-mediated vascular dilatation of the forearm, findings that suggest effects on pulmonary and systemic endothelial function.

The **purpose of this study** is to determine, in healthy subjects, the effects of inhalation concentrated outdoor UFP on pulmonary and systemic endothelial function, heart rate variability, and inflammation.

We will test the **primary hypotheses** that inhalation of ambient outdoor UFP 1) transiently reduces the pulmonary capillary blood volume and 2) blunts flow-mediated dilatation of the brachial artery. The **secondary hypotheses** are that exposure of these subjects to ambient UFP results in: 1) altered cardiac autonomic balance; 2) pulmonary and systemic inflammation; and 3) vascular effects comparable to those seen previously in our laboratory with carbon UFP.

II. PURPOSE OF THE STUDY AND BACKGROUND

Epidemiological studies have shown consistent statistical associations between ambient particle concentrations and adjusted mortality and morbidity rates (Pope et al. 2004). The United States Environmental Protection Agency (EPA) has estimated that approximately 60,000 excess deaths occur each year as a result of particulate air pollution and a majority of these deaths are from cardiovascular causes, including myocardial infarction (MI), sudden death, and congestive heart failure. Both the EPA (U.S. Environmental Protection Agency 2004) and the National Academy of Sciences (National Research Council 1998) have identified that the determination of the biological mechanisms involved in these deaths is a high priority research need.

Ambient particle exposure may influence cardiac function and blood coagulability. Panel and epidemiological studies have shown associations between ambient fine particle concentrations and reductions in heart rate variability (HRV) (Gold et al. 2000; Pope 3d et al. 1999), increased risk for acute MI (Peters et al. 2001), increased cardiac arrhythmias (Peters et al. 2000), increased blood viscosity (Peters et al. 1997), increased levels of C-reactive protein (Donaldson et al. 2001; Seaton et al. 1999), and increased fibrinogen (Hilt et al. 2002; Pekkanen et al. 2000). However, not all studies have shown these effects.

Because there are more deaths from cardiovascular causes than from respiratory causes, most deaths attributable to air pollution are from cardiovascular causes, including MI, sudden death, and congestive heart failure (U.S. Environmental Protection Agency 2004). A recent large

epidemiologic study linking air pollution data from U.S. cities with cause-specific mortality, found that a 10 $\mu g/m^3$ increase in fine PM was associated with an 8 to 18% increase in cardiovascular causes of death (Pope et al. 2004). PM-related mortality was chiefly due to ischemic heart disease, dysrhythmias, heart failure, and cardiac arrest.

Seaton et al. (1995) have proposed that pollutant exposure induces a transient increase in blood coagulability as part of the acute phase response associated with inflammation, although no data exist to support the theory. We propose that, if exposure to UFP causes airway inflammation, there will be activation of local endothelial cells and circulating leukocytes, and release of tissue factor by circulating monocytes, minimally (but measurably) activating the coagulation cascade. The combined effect of these processes could precipitate adverse cardiac events in individuals with critical coronary lesions.

Endothelial dysfunction is the underlying and earliest abnormality in atherosclerotic vascular disease (Quyyumi 1998). Physiological studies have demonstrated that vasodilation in response to physiological stimuli is mediated predominantly by nitric oxide (NO), and that abnormal endothelial function or responsiveness is a functional NO deficiency state, allowing vasoconstrictors such as endothelin to predominate. Endothelial NO is reduced in cigarette smoking, diabetes, hyperlipidemia, and atherosclerosis (Quyyumi 1998). Endothelial function is abnormal in these states, and this dysfunction precedes the development of atherosclerotic plaques.

We have completed four studies of exposures to elemental carbon UFP with healthy volunteers, and one study with asthmatics. In the first study, 12 healthy volunteers were exposed for 2 hours at rest to ultrafine carbon particles at a concentration of 10 $\mu g/m^3$, or approximately 2 x 10^6 particles/cm^3 (UPREST, RSRB #8006). Respiratory symptoms, spirometry, blood pressure, pulse oximetry, and exhaled NO were assessed before and at intervals after the exposure. Sputum induction was performed 18 hours after exposure. Continuous 24-hour, 12-lead Holter monitoring was performed on the day of the exposure and analyzed for changes in heart rate variability and repolarization phenomena. Repeated measures of ANOVA showed that very few significant exposure effects were observed out of a large number of endpoints, no more than would be expected by chance alone.

A subsequent study examined the effects of two concentrations of ultrafine carbon particles, 10 and 25 $\mu g/m^3$, with intermittent exercise during exposure (Dose- Response to Ultrafine Carbon Particles with Exercise (UPDOSE, RSRB #8293). As in the resting study, there were no symptoms, changes in lung function, or evidence for airway inflammation (induced sputum or exhaled NO) associated with the exposures. Pulse oximetry showed a clinically insignificant (about 1%) but statistically significant decrease in oxygen saturation in women after exposure to 25 $\mu g/m^3$ UFP. Studies of blood leukocytes showed early reduction in blood monocyte expression of intercellular adhesion molecule-1 (ICAM-1, CD54) in both men and women, and later reductions in the percentage of blood monocytes in women that were greatest 21 hours after exposure (Frampton et al. 2005). There was no significant change in the total white blood cell count. There was increased lymphocyte expression of CD25 (an epitope of the IL-2 receptor, a marker of activation), again only in women. Taken together, these findings are most consistent with particle effects on vascular endothelium, leading to subtle changes in pulmonary capillary perfusion, sequestration of monocytes that are expressing higher levels of ICAM-1 (leaving the low-expressing cells in the circulation), and shifts in circulating lymphocyte populations. Furthermore, we observed a blunting of the post-exercise increase in

the QTc interval of the electrocardiogram (ECG) in association with both UFP concentrations, suggesting subtle particle-induced changes in cardiac repolarization.

In our third study of healthy volunteers, (UP50, RSRB # 9336), 16 subjects underwent a 2-hour mouthpiece exposure to either filtered air or carbon UFP at a concentration of 50 $\mu g/m^3$. Subjects exercised on a bicycle ergometer for 15 minutes of each 30 minutes during the 120 minutes of exposure, for a total of four 15-minute exercise periods. Measurements included spirometry, the pulmonary diffusing capacities for carbon monoxide and nitric oxide (DLCO and DLNO, respectively), ECG, Holter monitoring, forearm blood flow test, serum markers of inflammation, and pulse oximetry. This was the first study in which DLCO was included as an outcome measure. Data demonstrated small but statistically significant decreases in DLCO and mid-expiratory flow rates at 21 hours after exposure (Pietropaoli et al. 2004a).

Sixteen subjects with mild asthma were exposed to air and to 10 $\mu g/m^3$ carbon UFP, using the same protocol as in the UP50 study (UPASTHMA, RSRB # 8849). The asthmatic subjects did not experience symptoms or changes in lung function (Pietropaoli et al. 2004a). Blood studies confirmed our observations in healthy subjects, with small but significant decreases in leukocyte adhesion molecule expression (Frampton et al. 2005). No significant effects on the QTc interval were observed. We also found no effects on blood coagulation (Pietropaoli et al. 2004b).

We have recently completed another study, (UPDLCO, RSRB # 10076), to confirm the decrease in DLCO with UFP in healthy human volunteers, and to compare UFP with an equal mass dose of larger carbon particles that have a smaller surface area. Twelve healthy adults were exposed to air, 50 ug/m^3 UFP (count median diameter 30 nm, particle number 1 x $10^7/cm^3$, surface area 750 m^2/g) or 75 ug/m^3 fine particles (count median diameter 300 nm, particle number 1 x $10^3/cm^3$, surface area 7 m^2/g) by mouthpiece for 2 h, with intermittent exercise. Exposures were randomized, double-blinded and separated by at least 2 weeks. Effects on oxygen saturation, DLCO and DLNO were assessed before and at intervals after exposure. We again saw a reduction in DLCO 21 hours after exposure which returned toward baseline at 48 hours after exposure. A similar reduction was seen with the fine carbon particles, although the reduction was not statistically significant. We estimated the pulmonary capillary blood volume (Vc) by taking advantage of the known differences in diffusivity between carbon monoxide and NO. This calculation incorporates the DLCO, DLNO, and blood hemoglobin. Both UFP and fine particle inhalation significantly reduced Vc.

None of these studies showed particle effects on lung function, symptoms, heart rate, or cardiac arrhythmias. Subjects were unable to tell when they were breathing particles.

In summary, there is strong epidemiological evidence that exposure to ambient particles increases the risk of adverse cardiovascular events. Recent studies indicate acute effects on both pulmonary and systemic endothelial function, with induction of a mild procoagulant state. Our own studies indicate that inhalation of even an "inert" ultrafine carbon particle has circulatory effects, in the absence of significant airway inflammation.

Based on the above observations, we hypothesize that exposure to carbon UFP presents an increased burden of ROS to the vascular endothelium, depletes NO, and impairs endothelial function in both the pulmonary and systemic vascular beds. We further hypothesize that outdoor UFP will have effects similar to those we have observed for laboratory-generated carbon particles. We will therefore compare the effects of UFP in this protocol with the effects seen in

our previous studies of carbon UFP. In addition, we will test the hypothesis that the potential for the UFP aerosol to generate ROS correlates with the vascular effects.

III. CHARACTERISTICS OF THE RESEARCH POPULATION

A. Number of Subjects:

Twenty subjects will be needed to complete the study. We estimate approximately 30 subjects will be screened to achieve this.

B. Gender of Subjects:

There will be equal numbers of male and female subjects.

C. Age of Subjects:

The age of the subjects will be 30 to 60 years.

D. Racial and Ethnic Origin:

No subject will be excluded from this study on the basis of gender, race, or ethnic group. Women of childbearing potential will not be excluded unless they are pregnant or breast-feeding.

The Table below shows the planned enrollment of subjects according to the categories indicated. This reflects the population distribution in the Rochester area. This representation will be achieved through differential acceptance of volunteers, and if necessary, through specific recruitment of under-represented groups.

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not Hispanic	Hispanic	White, not Hispanic	Other or Unknown	Total
Female	0	1	1	1	7		10
Male	0	1	1	1	7		10
Unknown							
Total	0	2	2	2	14		20

E. Inclusion Criteria:

Volunteers will be healthy, never-smokers with normal spirometry based on the standards published by Morris and co-workers (44).

F. Exclusion Criteria:

1. Any history of habitual smoking.
2. Marijuana smoking within the past 5 years.
3. Pregnancy.
4. Any history of significant organ impairment, chronic respiratory disease, ischemic heart disease, active psychiatric disorder or current drug or alcohol abuse.
5. Occupation involving regular, heavy dust or particle exposure, such as welding, mining, foundry work.
6. Abnormal spirometry (FEV_1 or FVC < 80% predicted; FEF_{25-75} < 60% predicted.
7. Subjects with atopy or allergic rhinitis will not be excluded as long as they do not require regular treatment with antihistamines or systemic steroids.
8. Subjects need to be off of the following medications (table) during and for the indicated interval before the study. Use of other medications will be considered on an individual basis. Subjects will not be asked to discontinue prescription medications for the purposes of this study.

Subjects must be able to avoid the medications listed in this table for the time indicated in each column heading:

1 MONTH	1 WEEK	1 DAY TO 1 WEEK
Systemic steroids such as prednisone	Non-steroidal anti-inflammatory drugs such as ibuprofen, naproxen, aspirin Supplemental vitamins Antihistamines Anti-oxidants Fish oil Niacin Arginine Over-the counter decongestants	Sildenafil (Viagra) (36 hours) Vardenafil (Levitra) (36 hours) Tadalafil (Cialis) (87 hours)

G. Vulnerable Populations:

Students at the University of Rochester and other area campuses will be allowed to participate in this study; however, students and staff specifically supervised or evaluated by one of the investigators will be excluded.

H. Restrictions on Recruited Subjects:

Subjects will be asked to avoid caffeine-containing beverages on study days. They will also be placed on a low-nitrate diet developed at the National Institutes of Health (NIH), beginning with breakfast on the day before each exposure, and ending after the final set of post-exposure measurements.

Subjects will not be studied within six weeks of a respiratory infection.

IV. METHODS AND PROCEDURES

A. Protocol

Each subject will have two exposures and a total of 7 visits, spanning about 6 weeks, with each exposure separated by at least 3 weeks.

Visit 1 is a screening day. Subjects will provide written informed consent; complete a standardized questionnaire for assessment of respiratory symptoms, medical and smoking history; and undergo a physical examination, spirometry, measurement of lung volumes, and a 12-lead ECG to exclude clinically evident coronary artery disease. At the time of screening, blood will be obtained for CBC, SMA-14, fasting lipid profile, and hemoglobin A1C. Premenopausal women will be screened for pregnancy. This visit is estimated to take 2 hours.

On a separate day (**Visit 2**), subjects will be admitted to the Clinical Research Center (CRC) at 12:00 on the day before the first exposure. They will have eaten a low-nitrate breakfast at home that morning, and will be given lunch on the CRC. At 13:00 they will undergo the following procedures: pregnancy test for pre-menopausal women, vital signs (blood pressure, heart rate and pulse oximetry), a symptom questionnaire, 12-lead Holter heart monitor attached with a resting recording for 10 minutes, forearm blood flow test, DLNO, DLCO, measurement of airway NO dynamics, phlebotomy (50 ml), and spirometry. These procedures will take approximately 2 hours. Subjects will be given dinner that evening, and will stay on the CRC overnight.

The following morning the subject will have breakfast at 06:30. At 07:15 the subject will be transported by wheelchair to the human exposure facility in the basement of the Kornberg Medical Research Building. The exposure to either clean, filtered air or concentrated UFP will occur from 07:30 to 09:30, inside an air-tight, air-conditioned plexiglas chamber (6 x 5 x 3.5 feet, 98 cubic feet). The chamber will be at negative pressure, approximately 12 cm H_20 relative to atmospheric, which is necessary to draw air flow through the concentrator. Exposures will be at rest. An investigator or trained technician will directly observe the subject throughout the exposure.

Immediately after the exposure, vital signs will be recorded; the subject will complete a symptom questionnaire, and will then be transported by wheelchair back to the CRC. Lunch will be provided at 12:00. At 13:00, the same procedures will be performed as on the previous day: vital signs (blood pressure, heart rate, and pulse oximetry), a symptom questionnaire, resting heart recording for 10 minutes, forearm blood flow test, DLNO, DLCO, measurement of airway NO dynamics, phlebotomy (50 mL), and spirometry. At the completion of these tests, the subject will leave the CRC, with the Holter monitor continuing to record overnight at home. The subject will be given an activity diary to complete at home, and instructions for the low-nitrate diet.

On **Visit 3** the subject will return the next morning at 08:00 after a low-nitrate breakfast at home. The subject will be allowed to remove the Holter electrodes at home and shower or bathe prior to this visit. Upon arriving, the activity diary will be collected, the Holter monitor will be re-attached with continuous recording, and the subject will undergo the same sequence of tests as on the previous day: vital signs (blood pressure, heart rate, and pulse oximetry), a symptom questionnaire, 12-lead Holter heart monitor attached with a resting recording for 10 minutes, forearm blood flow test, DLNO, DLCO, measurement of airway NO dynamics, phlebotomy (50 ml), and spirometry. The subject will be given another activity diary, and will go home with continued Holter monitoring.

On **Visit 4** the subject will return the following morning at 08:00, approximately 48 hours after exposure, for the same procedures as on Visit 3. The diary will be handed in, and the Holter monitor leads will be removed at the end of this visit.

On **Visit 5**, at least three weeks after Visit 2, the subject will return for the alternate exposure (air or UFP). Procedures on this day and on **Visits 6 and 7** will be identical to Visits 2, 3, and 4. Completion of Visit 7 will conclude the subject's participation in the study.

B. Procedures

1. Exposures

Our previous controlled clinical exposures evaluating potential adverse effects of airborne UFP have focused exclusively on laboratory-generated UFP composed of elemental carbon. As described above, we observed significant effects in healthy subjects, even with those surrogate particles. Among the reasons for using laboratory-generated particles were: 1) the reproducibility of the laboratory-generated ultrafine carbon aerosol, in contrast to the daily variability in outdoor particle concentration and composition; 2) for these initial human studies of UFP exposure, the desire for safety reasons to use a likely "benign" UFP of simple chemical composition ; and 3) the lack of a well-characterized system for concentrating the normally very low mass concentrations of ambient UFP for use in inhalation exposures. We have now shown that exposures to carbon UFP at concentrations as high as 50 $\mu g/m^3$ ($\sim 10^7$ particles/cm^3) do not induce symptoms or adverse clinical effects. However, the evidence for subtle vascular functional effects with elemental carbon UFP suggests a mechanistic link between UFP exposure and vascular effects. We are therefore

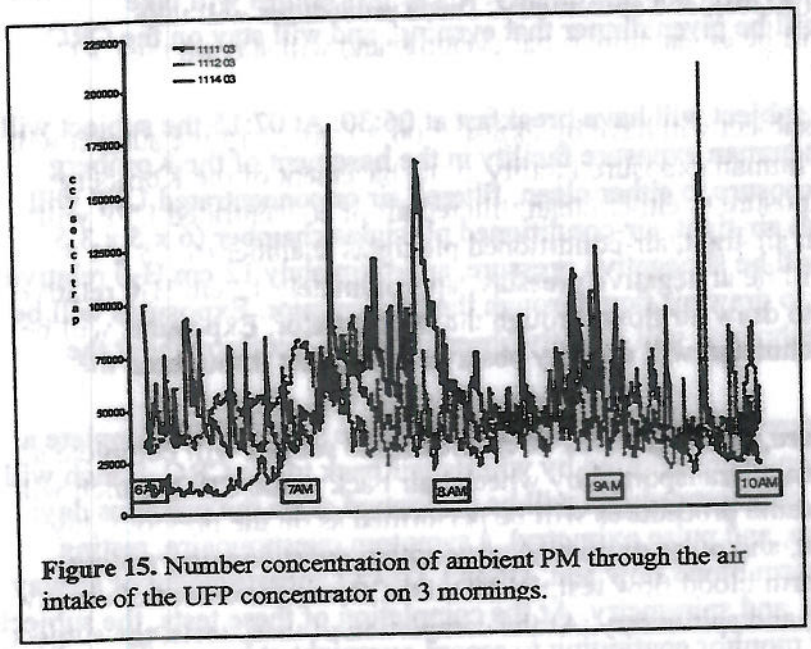

Figure 15. Number concentration of ambient PM through the air intake of the UFP concentrator on 3 mornings.

committed to extending these studies to ambient UFP, now that the concentrator technology has been developed.

The final version of the Harvard Ultrafine Concentrated Ambient Particle System (HUCAPS) (Demokritou et al. 2002) has now been installed in the basement of the MRBX, adjacent to the animal exposure facility on one side, and the newly constructed human exposure facility on the other side. The new HUCAPS will be used for both human and animal exposure studies; the animal studies are already underway. The human exposure facility is dedicated solely to human studies, is temperature- and humidity-controlled, and houses the Plexiglas exposure chamber. The output of the concentrator is ducted through the wall from the HUCAPs; overflow and exhaled aerosol will be vented outdoors via an exhaust system already in use for animal exposures.

Ambient air will be taken in from a street adjacent to the exposure room (Kendrick Road) via a 12-inch diameter duct system. We have measured UFP concentrations through this air intake on Kendrick Road over the course of several days (Figure 15). Substantial particle numbers are present in the morning hours; this is the aerosol concentration that will be concentrated. Our 2-hour exposures will take place in the morning to coincide with peak traffic-related particle counts, and thus allow us to specifically target traffic-related UFP. The HUCAPS concentrates UFP about 20-fold, which would provide exposures to particle numbers up to $10^6/cm^3$. This is in the range of peak particle numbers measured in the cab of a truck on a busy highway (Kittelson et al. 2001), and is an order of magnitude lower than the particle number used in our most recent carbon UFP study ($10^7/cm^3$). These initial concentrator studies will be conducted at rest, which will further reduce particle intake.

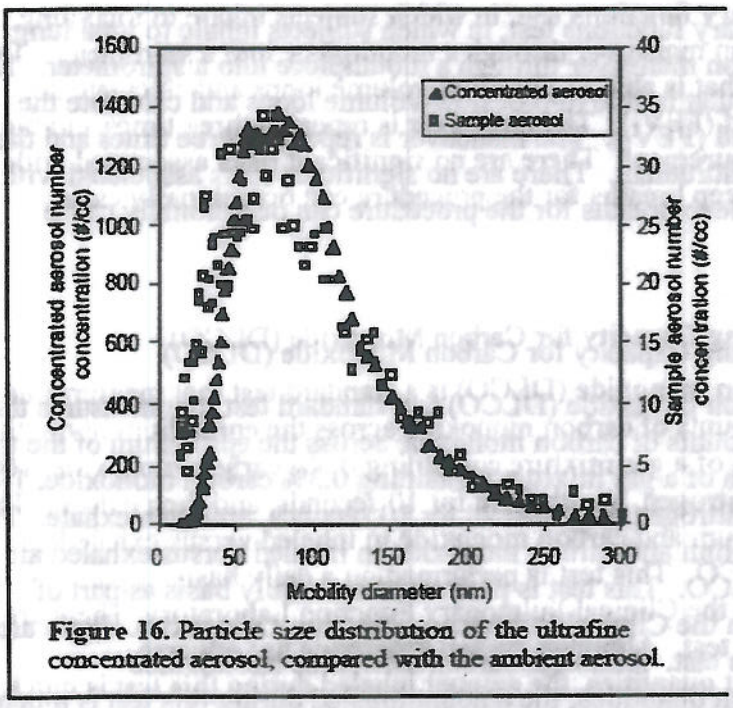

Figure 16. Particle size distribution of the ultrafine concentrated aerosol, compared with the ambient aerosol.

The HUCAPS concentrates without significant distortion of the original particle size. The underlying principles are 1) condensational growth to supermicron sizes, followed by 2) efficient concentration for particles larger than 1 μm by two stages of virtual impaction with low particle losses and low pressure drop, and finally 3) restoration of the original size distribution by reheating the condensationally grown and concentrated particles. Figure 16 shows results from tests performed jointly by members of the Rochester and Harvard PM Centers in July 2005, comparing the particle sizes of incoming ambient UFP with those of the resulting concentrated aerosol exiting the concentrator. There is excellent agreement between the two particle size distributions. In addition, an Aerosol Time-of-Flight Mass Spectrometer (ATOFMS) (Su et al. 2003) was used to characterize chemically the individual ambient and

concentrated particles in real time. The results confirmed that the HUCAPS concentrates ambient UFP without any significant change in the size distribution.

The particle concentrating process does, however, cause minor changes in particle composition. The single particle analyses showed that elemental carbon particles in the aerosol had a slight uptake of semivolatile organic compounds, due most likely to the uptake of gas phase volatile compounds during condensational growth (Su et al. 2004). This is a well-known physical phenomenon. We do not know at this point whether this small change in composition alters particle reactivity or toxicity, although this seems doubtful. Overall, the tests performed collaboratively by the Rochester and Harvard PM Center members confirmed that the HUCAPS is a versatile device suitable for toxicological inhalation studies.

Particle number and mass concentrations and size distributions will be continuously monitored for the un-concentrated outdoor air, and for the concentrated aerosol in the exposure chamber. A portion of the concentrator output will be diverted to sampling filters, for subsequent measurement of chemical composition and ROS generation of the concentrated aerosol. The particle concentration and composition will vary daily, and these exposure-day measurements will be used to construct dose-response relationships, and determine particle sources based on chemical composition.

2. Spirometry

Spirometry is a routine pulmonary functions test, in which subjects inhale to total lung capacity and perform a forced exhalation maneuver through a mouthpiece into a spirometer. The spirometer is connected to a computer that is able to plot flow-volume loops and calculate the volume of air exhaled in the first second (FEV_1). The maneuver is repeated three times and the largest of the values is used as the measurement. There are no significant risks associated with performing spirometry. Taking three deep breaths for the procedure can occasionally cause lightheadedness.

3. Measurement of Diffusing Capacity for Carbon Monoxide (DLCO)

The diffusing capacity for carbon monoxide (DLCO) is a standard test that measures the ability of the lungs to take up trace amounts of carbon monoxide across the epithelium of the tiny air sacs. Subjects inhale a single breath of a gas mixture containing 0.3% carbon monoxide, 10% helium, 21% oxygen, and the balance nitrogen, breath-hold for 10 seconds, and then exhale. The differences in the concentrations of helium and carbon monoxide in inhaled versus exhaled air allow the computer to calculate the DLCO. This test is performed on a daily basis as part of complete pulmonary function testing in the Clinical Pulmonary Function Laboratory. There are no significant risks associated with this test. Although carbon monoxide has adverse consequences when inhaled in sufficient quantities, the amount inhaled during this test is much less than that inhaled while smoking a cigarette, and has no physiological effects.

4. Measurement of Lung Volumes

Measurement of lung volumes is also part of routine pulmonary function testing, and will be performed once prior to the first exposure. The subject enters a body plethysmograph, a

Plexiglas box with dimensions similar to a phone booth. By panting against a shutter, thoracic gas volume is measured non-invasively using an application of Boyle's law. The test requires up to 15 minutes and is a standard clinical test performed in pulmonary function laboratories.

5. Measurement of Diffusing Capacity for Nitric Oxide (DLNO)

The diffusing capacity for nitric oxide (DLNO) is measured using the same principles as for DLCO. The subject inspires to total lung capacity with gas enriched with 1 to 10 ppm NO, followed by a 3- to 5-second breath hold and then exhales at 0.5 to 1.0 liters per second. This is repeated two times.

6. Measurement of Airway Nitric Oxide (NO) Dynamics

NO in exhaled air has been measured in our previous protocols. The entire procedure requires approximately 30 minutes for completion. Individuals breath-hold for 10 seconds and then exhale at six constant expiratory flow rates, which range from approximately 10 to 1,000 ml/sec. NO is measured in the exhaled breath during these maneuvers. Exhalations at each flow rate are performed in duplicate.

Ingestion of leafy green vegetables high in nitrates (e.g., lettuce, spinach) temporarily elevates exhaled NO concentrations by non-enzymatic reactions in the saliva (Zetterquist et al. 1998). To avoid this as a confounding factor, subjects will be placed on a NIH-developed low nitrate diet and asked to follow it at breakfast on study days. The lunch provided in the CRC will conform to the diet.

7. Phlebotomy

We hypothesize that the inhalational challenge with concentrated UFP will result in slight increases in blood markers of inflammation, and subtle alterations in hemostatic factors. It will be important to compare these measurements with those we made for the carbon UFP studies, in which we have not seen effects on systemic inflammation or coagulation. These changes will not be clinically significant in subjects without overt cardiovascular disease, but will be measurable using sensitive markers. We will compare plasma levels of markers at intervals following inhalation to the baseline levels in individual patients to evaluate the extent and time course of activation.

During the development of an inflammatory response, both circulating leukocytes and the endothelial cells lining blood vessels become activated and express markers on their surfaces that are involved in the process of leukocyte emigration to sites of inflammation. Some of these markers are shed from the cell surface and can be measured in serum. We will measure a panel of soluble adhesion molecules in serum obtained at various times before and after exposure, and will also measure the expression of adhesion molecules and activation markers on circulating leukocytes using flow cytometry. In addition, we will measure indicators of the systemic acute phase response, activation of coagulation, total ROS and products of NO metabolism in blood. These blood markers may prove to be sensitive indicators of airway inflammatory, as well as systemic, effects of pollutant exposure.

Phlebotomy will be performed using standard techniques and universal precautions, by one of the investigators, one of the fellows in the Pulmonary and Critical Care Unit, or by one of the laboratory technicians in the Unit who has been trained to perform the procedure. The amount of blood to be withdrawn at each blood draw will be 50 ml or less. The total amount of blood drawn over three days for any one exposure session will be 250 ml or less, with a maximum of 500 mL for the entire study (over more than 6 weeks). The techniques used for phlebotomy will be identical to those in our previous studies.

Serum and plasma samples from consenting subjects will be retained for future research (non-genetic), using an alpha-numeric code to protect subject identity. They may be contacted in the future to obtain a separate consent for genetic testing.

8. Flow-Mediated Dilatation (FMD) of the Brachial Artery

Detection of changes in endothelial function is central to this proposal, because abnormal endothelial function is clearly and closely linked with vascular disease and cardiac events (Quyyumi 1998). The response of the forearm circulation to flow-mediated dilatation (FMD) is mediated by endothelial release of NO. Depressed FMD is considered to be secondary to failure of shear stress-induced NO release because of endothelial cell injury, and/or NO inactivation by ROS. In patients with risk factors for atherosclerosis, there is evidence for reduced vascular NO bioavailability and for increased production of superoxide anion. Patients with reduced FMD are at increased risk for cardiovascular events, and measures that improve FMD also reduce cardiovascular risk. A finding of transient reduction in FMD following inhalation of UFP would suggest the potential for increased cardiovascular risk with repeated or long-term exposures.

High-resolution ultrasound imaging will be used to measure brachial artery diameter and flow in response to endothelium-dependent and endothelium-independent vasodilatation (Celermajer et al. 1992; Lieberman et al. 1994). Patients will be studied after remaining in a supine position for at least 10 minutes in a quiet temperature-controlled room located in the CRC. ECG leads will be placed on the chest for heart rate and rhythm monitoring. A small cuff is placed on widest part of the left forearm, 1-2 cm distal to the antecubital fossa. The left arm of the subject is extended, angled away from the body; the elbow is positioned with the hand rotated so the thumb points toward the ceiling. A Sequoia 512 (Acuson Computed Sonography, Mountain View, CA) ultrasound system with 15L transducer will be used to image the brachial artery. A custom preset protocol will be used. The section of the brachial artery approximately 2 cm above the antecubital fossa is located with a medial approach and the position is maintained throughout the exam. Color Doppler is used as a guide if necessary. The transducer will be aligned with the long axis of the brachial artery so that at least a 1.5 cm-long target area is visible. The transmit focus and receiver gain settings will be used to optimize imaging of the lumen/arterial wall interface. Two-dimensional (2D) images of the vessel will be stored to disk in cine-loop format. Each digital cine-loop will have a 3-second segment and at least 2 loops will be obtained at each stage. Pulse-wave spectral Doppler images will also be digitally stored to computer disk to calculate blood flow rates.

Endothelium-dependent vasodilatation: Forearm blood flow will be controlled using a sphygmomanometer cuff to ensure rapid and reproducible forearm occlusion/reperfusion. Brachial artery diameter and flow will be measured before and immediately following 5 minutes of supra-systolic forearm cuff occlusion (250 mmHg).

Endothelium-independent vasodilatation: After a second 10-minute rest period endothelium-independent vasodilatation will be tested using sublingual nitroglycerine (0.4 mg). Brachial artery diameter and flow will be measured before and after occlusion as previously.

Testing procedure summary:

- Brachial artery diameter and flow images are saved to disk. Resting vitals are obtained.
- An on-screen timer is started coincident with inflation of the forearm cuff to 250 mmHg.
- After 5 minutes of occlusion, reactive hyperemia is induced by the quick release of the cuff.
- Brachial artery Doppler flow images are saved to disk 0 to 30 seconds following cuff release. This is followed by brachial artery R-wave gated digital images from 45 to 75 seconds after release.
- After a 10-minute rest period, resting brachial artery diameter and flow images are saved to disk and the vital signs are again obtained.
- The on-screen timer is restarted coincident with placing one 0.4 mg nitroglycerine tablet under the tongue. Three minutes later, brachial artery diameter and flow images are saved to disk and vital signs are repeated.

Analysis: The brachial artery diameter will be measured using custom software that averages measurements along a 1-cm target region from the stored 2D digital images (LabView, Austin, Tx). The mean vessel diameter will then be calculated. In addition, the mean single-beat spectral Doppler flow velocity-time integral (VTI) will be calculated from 3 cardiac cycles to determine the absolute and relative brachial artery volumetric flow.

Absolute brachial artery blood flow (cc/min) is calculated as follows:

$$\text{Flow Rate} = \text{HR} \times \pi \times \frac{(\text{Mean Diameter})^2}{4} \times \text{VTI}$$

The relative change in vessel diameter and volumetric flow will be calculated as follows:

$$\text{Relative Change} = \frac{\text{Hyperemia Measurement}}{\text{Resting Measurement}} - 1$$

An important factor is appropriate standardization of experimental conditions, because many variables influence endothelial function. Subjects will be studied after a light breakfast, always supine, at the same times of day for each exposure. Subjects will not ingest caffeine on the study days, and will avoid large fatty meals in the 24 hours prior to study. This will be facilitated by admitting subjects to the CRC the day before each exposure.

9. Cardiac Monitoring

Cardiac monitoring will involve 48-hour ambulatory monitoring of the 12-lead ECG, a technique currently in use in our facility. The 24-hour ambulatory recording will be acquired continuously for 48 hours, beginning shortly after arrival on the CRC on the day before exposure (Visits 2 and 5), and ending after post-exposure followup (Visits 4 and 7). Subjects will be asked to rest quietly for 10 minute periods (to obtain high-resolution recordings) at the following times: the day before exposure, about 4 hours after exposure, and on return visits each of the next 2 mornings.

The 12-lead ECG continuous recording (acquired in digital format using the Mortara 12-lead Digital Holter System) will serve to evaluate morphology (ST segment, T wave), duration (QT interval), and dispersion (inter lead QT dispersion) of repolarization, as well as repolarization variability (microvolt T wave alternans and T wave variability) in controlled supine conditions. The recording will also allow us to evaluate the dynamic pattern of ECG parameters with particular emphasis on HRV, QT and R-R relationships, and repolarization morphology assessed during exercise and during post-exposure activities. The prolonged post-exposure ambulatory monitoring will evaluate night-time changes in HRV and repolarization adaptation.

Pulse oximetry will be included as part of the vital signs measurements.

C. Data Analysis and Data Monitoring

The basic design for each phase of this project consists of a standard two-period, cross-over design in which each subject receives both treatments. The order of presentation is randomized, with half the subjects receiving each of the two possible orders. For some endpoints repeated measurements will be made at uniform intervals after exposure to each treatment. The standard analysis for continuous endpoints is a repeated measures analysis of variance (ANOVA). In these analyses, order of presentation is the between-subjects factor and treatment (and time for when there are repeated measurements) are within-subject factors. The analysis includes tests for period and carry-over effects. The ANOVA model and its interpretation have been described by Wallenstein and Fisher (Wallenstein and Fisher 1977). Each ANOVA will include an examination of residuals as a check on the required assumptions of normally distributed errors with constant variance. If these assumptions are not satisfied, data transformations (for example, square-root transformation for cell counts) will be considered.

The effects of UFP characteristics on the vascular responses will be assessed by multiple analysis of covariance. The dependent variables in these analyses will be measures of pulmonary and systemic endothelial function and blood markers of activation of the coagulation cascade. A total of 3 predictor variables have been identified: concentrated UFP oxidant capacity, mass, and number, measured on each exposure day. Multicollinearity may be an issue in these analyses. This will be checked using variance inflation factors. In particular we will check the correlations among the 3 particle parameters. Redundant variables will be combined or removed from the analysis. Each analysis will include an examination of residuals as a check on the required assumptions of normally distributed errors with constant variance. If violations are found, data transformations will be considered.

1. Sample Size

Using the conventional 5% level of significance and 80% power with a paired design, detectable effect sizes, as a percent of the baseline mean, were determined for sample sizes of 10 to 30 subjects (see table below). Data for measurements of baseline artery diameter, FMD, DLCO, and DLCO/DLNO were determined in our laboratories on healthy, nonsmoking subjects. Data for tissue factor (TF) activity were from diabetic patients without vascular complications, obtained from the literature (Zumbach et al. 1997).

Table: Effect sizes (% of baseline mean) detectable with n=10, 20, or 30.

		Number of Subjects		
Endpoint	**Mean±SD (n)**	**10**	**20**	**30**
Brachial artery diameter	0.42±0.08 (5)	0.46%	0.32%	0.26%
Flow-mediated dilatation (%)	11.2±10.7 (5)	1.56%	1.10%	0.90%
DLCO	28.84±6.89 (16)	6.6%	4.7%	3.8%
DLNO/DLCO	4.70±077 (16)	16%	11%	9.2%
Tissue Factor activity	0.11±0.04 (50)	20%	14%	10%

We have chosen a sample size of 20 subjects, which will be sufficient to detect a 14% change in TF activity, and to detect very small changes in artery diameter and FMD.

D. Data Storage and Confidentiality

Data will be recorded in bound laboratory books and transferred to and stored in a desktop computer using Excel software. Data will be transferred to the CRC server for preparation of SAS datasets. Only the investigators and technicians directly involved in data acquisition and analysis will have access to the data. Samples and data will be coded to protect the identity of the subjects. Computers and data books will be stored in a locked laboratory.

V. RISK/BENEFIT ASSESSMENT

A. Risk Category

This research presents greater than minimal risk to the subjects.

B. Potential Risks

1. Exposures

It is very unlikely that these exposures to concentrated outdoor particles will cause symptoms or clinically important effects. Similar studies are currently ongoing, using the same Harvard ultrafine particle concentrator, at the U.S. Environmental Protection Agency. They have nearly completed a study in healthy subjects and have found no adverse effects. Our previous studies of exposure to UFP at 50 $\mu g/m^3$, with intermittent exercise, were without adverse effects. This study will be conducted at rest, which will further reduce the particle dose. In a separate study ("Inhaled particle characteristics and early lung effects," RSRB #8126), healthy subjects have been exposed to ultrafine and fine zinc oxide particles at a concentration of 500 $\mu g/m^3$ without adverse effects. Previous human studies of exposure to fine carbon particles found no clinical effects of exposure to 250 $\mu g/m^3$ for 1 hour or 500 $\mu g/m^3$ for 2 hours (Beckett et al. 2005). The National Ambient Air Quality Standard for outdoor particulate matter in the air ($PM_{2.5}$) is 65 $\mu g/m^3$, averaged over 24 hours.

There is a small, theoretical risk of precipitating a cardiac event in persons with severe coronary artery disease. Therefore, subjects will have a screening ECG read by a cardiologist prior to exposure. Nevertheless, it is possible that subjects recruited for this study could have clinically silent cardiovascular disease, that might not be detected by the screening examination and ECG. We feel that the risk for precipitating a cardiac event in these exposures is extremely small, even for a subject with unrecognized coronary artery disease. First, the particles used in this study will be outdoor particles similar to what people breathe every day. The number concentrations will be higher than people usually inhale in Rochester, but will be similar to what people breathe when driving on a busy highway, or working in certain occupations.

Second, all exposures will be conducted at rest, as opposed to our previous studies in healthy subjects, which have generally involved exercise. Thus, the actual dose of particles to the lung will be lower in this study, and the cardiovascular stress of exercise will be absent. Finally, the risk of cardiovascular events associated with outdoor air particles is relatively small, and has required studies of millions of people to detect. While this risk is important from a public health standpoint, the net increase in risk for individuals on any given day of exposure remains very small. For example, Peters et al. (20) found in a study of residents in the Boston area, an odds ratio for MI of approximately 1.3 associated with an increase in air pollution from the cleanest days to the worst days. The age-adjusted incidence of first acute MI for adult males is approximately 10 per 1,000 person-years, or 10 per 365,000 person-days. Thus, according to the Peters data, the risk of having a heart attack related to the worst air pollution day in Boston would increase from 1 in 36,500 to 1 in 28,077. The risk would be lower for people without clinical evidence of coronary artery disease. Given that the exposures used in this study are for only 2 hours rather than a whole day, the risk of precipitating a cardiovascular event is extremely small.

2. Pulmonary Function Tests

These tests are performed on a daily basis in the pulmonary function laboratory, and are essentially without significant risk. Taking three deep breaths for spirometry can occasionally cause lightheadedness.

3. Measurement of DLNO

Inhalation of high concentrations of NO can be toxic to the lungs; however, the maximal inhaled level of 10 ppm of NO for only 1 to 3 breaths performed during this measurement is well below accepted toxic levels. For example, the threshold limits for exposure to nitric oxide published by the National Institutes of Occupational Safety and Health (NIOSH), the Occupational Safety and Health Administration (OSHA), and the American Conference of Government Industrial Hygienists is 25 ppm for 15 minutes to 8 hours per day (Lehnert 1993). Normal humans have 23 ppm of NO in the paranasal sinuses (Lundberg et al. 1994). We have found no adverse effects of performing these measurements in our previous protocols (RSRB #07121, 08006, 08293).

4. Measurement of Exhaled NO

The 25- to 30-second periods on a mouthpiece to measure NO may temporarily cause slight discomfort and drying of the mouth. Other potential risks are related to inhalation of NO, as explained in #3 above.

5. Phlebotomy

The risks of phlebotomy are minimal. Vasovagal syncope can occur, therefore, patients will be kept supine during phlebotomy. Subjects can also experience discomfort and/or bruising at the venipuncture site. More serious complications such as thrombophlebitis or infection, which can occur with indwelling intravenous catheters, are extremely unusual with simple phlebotomy.

6. Forearm blood flow test

Subjects may experience tingling of the hand or even brief numbness while the blood pressure cuff is inflated. Nitroglycerine can cause a brief headache, and may temporarily lower the blood pressure. However, it is generally well-tolerated, and is routinely used in research studies measuring systemic vascular responsiveness. Subjects will be supine during and following the administration of nitroglycerine.

C. Protection against Risks

The Principal Investigator will be responsible for safety monitoring, and for the reporting of any adverse events to the RSRB and the CRC.

The subject will be under direct observation by a trained investigator (usually the engineer running the exposure) at all times during the exposure. Monitoring facilities provide continuous measurements of particle concentrations. Symptoms and sensitive measurements of pulmonary function will be monitored before and after exposure. Exposures will be discontinued if any adverse symptoms occur. All subjects will remain in the CRC for approximately 6 hours

after exposure and symptoms will be assessed before discharge. Finally, subjects will return approximately 24 and 48 hours after each exposure to assess possible delayed effects.

The human exposure facility is located in the MRBX, a short walk from Strong Memorial Hospital. Physician availability is assured at all times.

D. Potential Benefits to the Subjects

There are no anticipated benefits to the subjects.

E. Alternatives to Participation

The alternative to participation in this study is not to participate.

VI. SUBJECT IDENTIFICATION, RECRUITMENT AND CONSENT/ASSENT

A. Method of Subject Identification and Recruitment

Twenty healthy, nonsmoking subjects will be recruited using advertisements on local bulletin boards and in area newspapers. Potential participants will call our Study Coordinator who will describe the nature of the study to the subject. If the subject meets the criteria for the study and expresses willingness to participate, an appointment will be made for a visit to the Pulmonary and Critical Care Division.

B. Process of Consent

Consent will be obtained by one of the investigators at the time of the initial visit. The consent form will be provided to the subject and the investigator will describe the study to the subject and will ask for and answer any questions. The subject will have the opportunity to take the consent form home to discuss it with family or advisors and to return with additional questions before deciding to participate. The consent form will then be signed by the subject and co-signed by the investigator. The subject will be given a copy of the signed consent.

C. Subject Competency

All subjects participating will be competent to provide consent, and competency will be determined by the investigator obtaining consent, using a brief mental status assessment.

D. Subject Comprehension

The subject's comprehension of the study will be assessed by the investigator, using questions designed to determine the subject's level of understanding of the study. After completing the presentation on the study and after the subject has read the consent form and asked questions, the subject will be asked to describe, in his or her own words, what will happen during the study.

E. Consent/Assent Form

Draft provided.

F. Documentation of Consent/Assent

The investigators are listed on the title page of the consent form. Those investigators who will obtain and document informed consent are listed below:

Mark W. Frampton, MD, Professor of Medicine and Environmental Medicine

Carol-Lynn Petronaci, MD, Senior Instructor in Medicine

Anthony Pietropaoli, MD, Assistant Professor of Medicine

Alpa Shah, MD, Fellow, Pulmonary and Critical Care Medicine

Mark J. Utell, MD, Professor of Medicine and Environmental Medicine

VII. FINANCIAL OBLIGATIONS AND INCENTIVES

A. Costs to the Subject

None.

B. Incentives for Participation

Subjects will be paid an honorarium of $100 after completing the screening visit, $400 after completing visit 4, $400 after completing visit 7, and $100 for completing the entire study, for a total of $1,000.

VIII. REFERENCES

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IRB Application

Date: Tuesday, May 30, 2006 1:23:21 PM - RSRB#: RSRB00013401 Print

1. Study Identification Information. Protocol & Measures

1.1 *** Study Working (short) Title:**
UPCON

1.2 *** Study Full Title:**
Exposure to Concentrated Outdoor Ultrafine Particles in Healthy Subjects (UPCON)

1.3 If the **Study Protocol** is available electronically, click **Add** to upload:

name	Revision	Modified Date
UPCON Protocol	0.02	4/5/2006 5:05 PM

1.3.1 * Does the study involve the administration of any assessments (surveys, questionnaires, diaries) or measures of human behavior? yes

If **Yes**, click Add to upload the **measure**(s)

name	Revision	Modified Date

There are no items to display.

1.4

Principal Investigator	HSPP/EPRP	Expiration Date
* Mark Frampton	10120204	2/28/2007

1.5 **Co-Principal Investigator(s):** (*Individuals who share full responsibility for the study with the Principal Investigator*)

Last	First	Organization	HSPP/EPRP No.	Expiration Date

There are no items to display.

1.6 **Sub-Investigator(s):** (*Individuals who assist PI or Co-PI in certain assigned aspects of the study*)

Last	First	Organization	HSPP/EPRP No.	Expiration Date
Pietropaoli	Anthony	Medicine	10710604	6/30/2007
Utell	Mark	Medicine	15421105	11/30/2006
Zareba	Wojciech	Cardiology	10970604	6/30/2007

If applicable, list all non-UR affiliate Investigator(s) include Name and Institution:

Submit a copy of the Human Subjects Investigator certification (or UR HSPP # for those institutions that do not provide such training.)

name	Revision	Modified Date

There are no items to display.

1.7

Study Coordinator	HSPP/EPRP	Expiration Date

	* Donna Speers	16770106	1/31/2007

1.8	* Who will be the primary contact for questions or correspondence? Principal Investigator If **other**, provide name and phone No.:

* Required field

2. Conflict of Interest

2.1.1	* Do any study personnel, spouses or dependent children receive or expect to receive **income for licensing discoveries** from the sponsor; or have an interest in a **patent, copyright or licensing agreement** whose value may be affected by this research? no
2.1.2	* Do any study personnel, spouses or dependent children serve in a **management capacity** or on the Board of Directors of the sponsor/company? no
2.1.3	* Do any study personnel, spouses or dependent children own any shares of **stock, stock options, partnership interest, or other ownership interest** in any privately or publicly held company whose value may be affected by this research (e.g., the sponsoring company)? no
2.1.4	* Do any study personnel, spouses or dependent children receive or expect to receive **financial compensation** from any company which may be affected by the outcome of this research (other than through a contract to the University to conduct this research)? no
	If *Yes* **to any of the above**: Include a management plan (or waiver) signed by your Dean. Possible conflicts of interest should be reviewed by the Department Chair first. **Attachment:** Note that disclosure in the consent form should be a part of the plan or waiver.
2.2	* Does the University have any conflicts of interest in this study? no
	If *Yes:* Explain:

form #

3. Source of Funding/Sponsorship (Grants and contracts **must** be submitted to the Office of Research and Projects Administration (ORPA).)

3.1	Please indicate Sponsor Type and Name:
	no **No Funding or Sponsor** no **Department Funding** **Department Name:** If other, please indicate: yes **Government Agency**

	* Donna Speers	16770106	1/31/2007

1.8	* Who will be the primary contact for questions or correspondence? Principal Investigator If **other**, provide name and phone No.:

* Required field

2. Conflict of Interest

2.1.1	* Do any study personnel, spouses or dependent children receive or expect to receive **income for licensing discoveries** from the sponsor; or have an interest in a **patent, copyright or licensing agreement** whose value may be affected by this research? no
2.1.2	* Do any study personnel, spouses or dependent children serve in a **management capacity** or on the Board of Directors of the sponsor/company? no
2.1.3	* Do any study personnel, spouses or dependent children own any shares of **stock, stock options, partnership interest, or other ownership interest** in any privately or publicly held company whose value may be affected by this research (e.g., the sponsoring company)? no
2.1.4	* Do any study personnel, spouses or dependent children receive or expect to receive **financial compensation** from any company which may be affected by the outcome of this research (other than through a contract to the University to conduct this research)? no
	If ***Yes* to any of the above**: Include a management plan (or waiver) signed by your Dean. Possible conflicts of interest should be reviewed by the Department Chair first. **Attachment:** Note that disclosure in the consent form should be a part of the plan or waiver.
2.2	* Does the University have any conflicts of interest in this study? no
	If *Yes:* Explain:

form #

3. Source of Funding/Sponsorship (Grants and contracts **must** be submitted to the Office of Research and Projects Administration (ORPA).)

3.1	Please indicate Sponsor Type and Name:
	no **No Funding or Sponsor** no **Department Funding** **Department Name:** If other, please indicate: yes **Government Agency**

Government Agency Name: NIH - National Institute of Environmental Health Sciences (NIEHS)
If other, please indicate:

Click Add to upload the government grant:
name description
Ultrafine Particle-Induced Oxidative Stress

Government Sponsored Grant Number:
RO1 ES013394

no **Foundation**
Foundation Name
If other, please indicate:

Click Add to upload the foundation grant:
name description
There are no items to display.

Foundation Sponsored Grant Number:

no **Industry Initiated**
Company Name:
If other, please indicate:

no **Industry: PI-Initiated**
Company Name:
If other, please indicate:

form #

7. Just-In-Time (JIT) Study Part 1	
7.1	* Is this a Just In Time (JIT) study? ○ Yes ◉ **No**

form #

6. Project Funding	
6.1	* Is the UR a sub-contractor of this grant? no
	If *Yes:* Name of principal grantee:
6.2	* Will the sponsor provide monetary support? [Industry sponsored studies may incur review fees.] yes
6.3	* Will the sponsor provide free drug and/or device? no

form #

8. Coordinating Center Studies, Concept Studies and Umbrellas No subject enrollment or access to subject data is allowed under a Coordinating center, Umbrella, or Concept study. All research activities involving human subject enrollment or subject data collection must be submitted separately for review.	
8.1	* Is this a multi-site study for which the University of Rochester is the Coordinating Center (i.e., subjects will enrolled by other "site" investigators)? no
8.2	* Is this a "Concept Study," i.e., the protocol is in development and no subjects will be enrolled nor will any data be collected until approval of the final protocol? no
8.3	* Is this an "Umbrella Study," i.e., does this study provide funding for sub-studies, but no subjects will be enrolled or data collected under this study itself? no

form #

9. Study Exemption	
9.1	* Do you think this study may qualify as exempt under one of the federally recognized exemptions? no
* Required field	

form #

49. Drugs, Devices and Biologics	
49.1	* Will the study be using drugs, devices or biologics? no

form #

61. Student Projects	
61.1	* Is this study a student project? no
	If *Yes:* Indicate what type of project: no Undergraduate Project no Master's Thesis no Doctoral Dissertation no Post-Doctoral Project no Resident/Fellowship no Medical Student Project **If other, please indicate below:**

form #

62. Institutional Oversight/Cooperative Approvals	
62.1	* Does this research proposal require review by any of the following University of Rochester committees? yes

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If *Yes* check all that apply. Unless otherwise noted, a copy of committee approval is required prior to RSRB approval.

no CTO [Clinical Trials Office] approval is required for hematology/oncology related studies proposed at the University of Rochester and its affiliates. (*Note: if the PI works for the Cancer Center, this option should be checked. Do not uncheck it.)*

yes GCRC [General Clinical Research Center] (If any part of the study is conducted at or uses any resources of the GCRC)

no Perinatal Research Committee (For studies involving pregnant women, women intending to become pregnant or newborns/infants in the normal nursery or neonatal intensive care unit at SMH or Highland Hospital. Contact Tel. 5-2520)

no ED Research Committee (For studies involving subjects in the Emergency Department or Emergency Medicine Faculty. Contact Tel. 5-8274)

no Institutional Biosafety Committee (IBC) (For the studies involving vaccines, gene therapy, viruses, serums, toxins or blood products. Contact Telephone: 5-3014)

no Surgical Pathology Approval (Required for use of slides or tissue from the Pathology Department)

no HURC/RDRC ('Radiation Safety') Committee approval required for research evaluation of unapproved or FDA-approved radiation-generating device; FDA-approved radiopharmaceutical given within product labeling or by an unapproved route; a radioactive drug that is FDA-approved or under an FDA-approved IND exemption. Contact Tel. 5-1473

no Other:
If other, please indicate below:

form #

1. GCRC - General Information	
(If you have any questions regarding section 72 (Parts 1, 2 and 3) please contact the GCRC directly at 275-6409)	
1.1	Click **Add** to upload a document contains the description of the project: UPCON SUMMARY The 'Description of the Project' section, consisting of 250 words or fewer in text format, should be written in lay language. Background, rationale for the project, study question(s), design, study population, and outcome measures should be included. This segment will be publicly available through CRISP, so it should not contain any proprietary or confidential material.
1.2	Has this protocol received funding as of this GCRC submission? yes
1.3	Projected start date: 2/1/2006
1.4	Does this study evaluate a disease that would qualify as a Rare Disease or condition as defined by the NIH? no
1.5	Expected duration of study from initial enrollment to completion of last subject: 1 year(s)

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1.6	Outreach plans to ensure appropriate representation of women and minorities is obtained: Targeted subject recruitment if necessary.
1.7	Are children (**under the age of 21**) are to be included? no If **No**, reason for exclusion of children - (Check all that apply) *Notes: if "No", at least one box must be checked.* no The research topic is irrelevant to children. no Laws or regulations bar the inclusion of children. no Information being sought is already available for children or will be provided in another study. yes A separate, age-specific study in children is warranted or preferred. no Not enough information is available regarding risk in adults to judge the potential risks in children. no Study is aimed at providing additional information on a previous all adult study. no Other special cases: the GCRC Advisory Committee will judge on an individual case-by-case basis. Describe:
1.8	Please justify **WHY** GCRC resources are needed: Multiple blood draws, overnight stay, low-nitrate diet, procedures to be performed on GCRC
1.9	Is this an AIDS-related study? no
1.10	Is this a multicenter trial? no
1.11	Is this a clinical trial? no
1.12	Data and Safety Monitoring Plan (DSMP): To help us review your protocol and determine where essential elements are included, please indicate on which page (or pages) they can be found in the protocol or grant application <u>Data Collection and Monitoring</u> Data to be collected (plan of study) and records to be kept (e.g. case report forms): Page(s) 7-15 Data Monitoring Page(s) 14-15 <u>Safety Monitoring/Adverse Events (AE) and Serious Adverse Events (SAE) Reporting</u>

	Who is responsible for monitoring safety (i.e. PI, safety monitor, DSMB, etc.) Pages 18 Plan for safety review; when SAEs and AEs reported, and to whom (e.g. RSRB, DSMB, GCRC, etc.) Pages 18 Does the protocol include an AE grading and attribution scale? If Yes, please indicate where this can be found. Page(s) Is there a Data and Safety Monitoring Board (DSMB) or Committee (DSMC)? If Yes, please indicate where the description of the composition of the board (names of members or specialties represented), its duties, and the DSMB/DSMC charter (if available) is located. Pages

form #

2. GCRC - Participant Projections

2.1 Please project the number of ***new*** subjects for each GCRC grant year (March 1st to February 28th of each year) and then provide the total number for the duration of the study.

	Inpatient	**Outpatient**
1st year	18	
2nd year	2	
3rd year		
4th year		
5th year		
Total:		

2.2 Are subjects to be studied as inpatients? yes

If **Yes**:
Per Subject: number of days per admission: 2

Number of admissions: 2
Total days required: 4

Will these inpatients be seen on the GCRC: yes

If No, where will they be seen?

2.3 Are outpatient visits included in study? yes

If Yes:
Per Subject: number of visits: 4

Approximate length of visit: 3 hours

Total hours per subject: 12

Will these outpatients be seen on the GCRC? yes

If No, where will they be seen?

form #

3. GCRC Services
Please indicate if the following services are needed:

3.1 NURSING SERVICES: yes

If Yes, check all that are needed:

yes Routine patient care (i.e. ht, wt, vital signs)

no Special cardiac monitoring

yes EKG

no Biopsies Type of Biopsy:

no Non-serial blood collections

yes Serial blood collections

no Heparin-locks

no IV lines

no Renal vein sampling

no IV infusions

	no 24 hour urine collections no Stool collections no Other, specify:
3.2	OTHER SERVICES: no If Yes, check as needed, and indicate the # of tests per subject no Bio-electrical impedance # /subject no Resting metabolic rate # /subject no Skin-fold measurement # /subject no DEXA scans # /subject (lumbar spine, hip, forearm, whole body, AP/lateral) no Other list:
3.3	NUTRITION SERVICES: yes If Yes, check all that are needed: no Regular meals or snacks no Standardized meals no Metabolic or constant diet no Computerized dietary analysis (i.e. food records, 24 hr recall, food frequency) no Pre-admission counseling for dietary control (i.e. high carbohydrate diet prior to OGTT) no Patient nutrition counseling/education yes Other (i.e. find appropriate nutrition assessment tools) List: Low nitrate diet
3.4	CORE LABORATORY SERVICES: no If Yes, please click the Add button below to list the test(s) Core Lab Listing There are no items to display.
3.4.1	Is special handling of samples required? yes If Yes, please explain: Centrifuge Other:

3.4.2	Is another lab also processing blood samples? yes If Yes, what lab is being used? SMH Clinical Laboratory
3.5	ANCILLARY SERVICES: yes If Yes: Please complete for all ancillary tests you are requesting to be paid from the GCRC grant by clicking on the Add button below. Ancillary Lab Listing [View] Are non-routine ancillaries requested (e.g. expensive drugs)? no If Yes, please justify why these are not to be charged to the funding agency. If the procedures are not listed, please include them with their associated cost:
3.6	INFORMATICS CORE: yes If Yes: A) Resources Requested: no Automated data entry/verification processes yes Database design, development, and management no Customized software creation and support no Streamlined data quality and management reporting no Internet-related solutions (i.e. Web applications, Web page development, Internet access to data) no Computer facility usage (3 PCs, network printer, scanners) no Other: B) Estimated data storage time: First entry of data: 2/1/2006 Analyses to be completed: 12/31/2007 C) Data collection for the project: yes To be initiated

	no In progress no Already completed If **No:** Please indicate which of the following will be utilized instead: no Investigator PC no Biostatistics no Drug Company no Other, describe:
3.7	BIOSTATISTICAL ASSISTANCE: no If **Yes:** no Project design no Data analysis no Other list: Biostatistician on the Project (if already contacted): Li-Shan Huang

form #

63. Study Site(s)	
63.1	* Will this research be conducted at Highland Hospital? no
63.2	* Will this research be conducted at any non-UR facilities? no If *Yes:* **List the name of facilities:** **Click Add to upload the IRB approval or the letter of cooperation:** name description There are no items to display.

form #

73. Cross Referencing Studies	
73.1	* Is this the same protocol as another study, but includes a different funding source? no If *Yes*, list RSRB#:
73.2	* Is this study similar to another study, but has protocol modifications? no

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	If *Yes*, list RSRB#:
73.3	* Is this a re-submission of a previously closed study? no If *Yes*, list RSRB#:
73.4	* Is this being funded or supported as part of another study? no If *Yes*, list RSRB#:
73.5	* Is this application part of a 'Five-year review'? no If *Yes*, List RSRB#:

form #

74. Use of Specimen(s)	
74.1	* Will this study use only stored or discarded tissue, blood and/or other biological specimen (s)? no
74.2	* Will any fetal tissue or stem cells be used in this study? no

form #

64. Subject Population
Note: Include **all** groups of subjects (for example children, parents/guardians, teachers, Providers, students, staff members) about whom information will be collected.

64.1	Will this study involve direct contact with study subjects? yes
64.2	Will this study involve access to subject records, specimens or information? yes
64.3	What is the Total Number of Research Subjects expected to be enrolled (or their records/specimens accessed) for this study? 20

	Number at UR Site(s)	Number at Non-UR Site(s)
Experimental Group (Treatment, Intervention, Interaction)	20	
Control Group (Non-Intervention, Placebo)		
Total Number of Subjects	20	

Gender/Minority Chart: UR Subjects (or Subject Records /Specimens Used)			
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	9	9	18
Ethnic Category Total of All			

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Subjects [1]	10	10	20

Racial Category			
American Indian/Alaska Native	0	0	0
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	1	2
White	8	8	16
Racial Category Total of All Subjects [1]	10	10	20

[1]The Ethnic Category Total of All Subjects must be equal to the Racial Categories Total of All Subjects.

Gender/Minority Chart: Non-UR Subjects (or Subject Records /Specimens Used) for which PI has responsibility			
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino			
Not Hispanic or Latino			
Ethnic Category Total of All Subjects [1]			

Racial Category			
American Indian/Alaska Native			
Asian			
Native Hawaiian or Other Pacific Islander			
Black or African American			
White			
Racial Categories Total of All Subjects [1]			

[1]The Ethnic Category Total of All Subjects must be equal to the Racial Categories Total of All Subjects.

64.4	Indicate the age ranges for subjects. Check all that apply and submit appropriate forms. (Note: exempt studies generally do not require written consent): no 0-6 years [Parent/Guardian Permission form required]

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	no 7-12 years [Parent/Guardian Permission form and verbal child assent script required] no 13-17 years [Parent/Guardian Permission form and written child assent form required] yes Adults: 18 years and older [Consent form(s) required] no Adults over age 89 [Consent form(s) required]

form #

75. Vulnerable Populations (to be Targeted)			
75.1	Check **all** that apply:		
	no Minors (Under 18 years) no K-12 students no UR students (under 18 years) no UR students (18 years and over)	no Employees no Pregnant women no Prisoners no Terminally ill (life expectancy less than 6 mos.)	no Nursing Home Residents no Limited or non-reader no Mentally compromised no Economically Disadvantaged no None
75.2	Are there enrollment restrictions based on:		
	Race? no Gender? no	Ethnicity? no Age? yes	Pregnancy? yes HIV Status? yes
	If *answer Yes* to **Pregnancy** or **HIV Status**, will you test prospective subjects for pregnancy or HIV Status? yes		
75.3	Provide scientific rationale for restricting or including any of the populations indicated in questions **75.1** or **75.2:** **The age range of 30-60 years was chosen to match that of our current study of people with type 2 diabetes (RSRB #10459). Elderly (over age 60) will be excluded because of the possibility of confounding health issues.**		
75.4	Describe how undue influence will be minimized for these subjects and how precautions will be used to protect the rights and welfare of these subjects: N/A		
75.5	Will decisionally impaired subjects or those of questionable capacity to consent be included?		

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	no If *Yes*: Explain briefly how capacity will be determined, who will make that determination, how the process will be documented and who will provide permission for incapacitated subjects: *Note: Provide a complete description of these procedures in the Protocol.
75.6	Does this study evaluate a disease that would qualify as a Rare Disease or Condition as defined by the NIH? no

form #

65. Non-English Speaking Part 1	
65.1	* Will any non-English speaking subjects be included in this study? no
65.2	If *Yes*: Have you included both English and non-English versions of all subject documents (i.e. consent forms, written questionnaires, information or recruitment letters)?

form #

66. Subject Recruitment or Use of Subject Records/Specimens

66.1 Check **all** methods of recruiting subjects (or methods of collecting subject data/specimens) for this study:

yes Poster	no Information Letter[1]	yes Brochure or Flyer
no Radio or TV Ad	no Email or Internet[1]	yes Newspaper
no Clinic or Private Practices[1]	no Referrrals[1]	no Medical Records[1]
no School/Day Care Records[1]	no Psychology sign-up bulletin	no Telephone Script
no Psychology Research Pool [PRP]		no Other: **If other, provide method below:**

Upload Recruitment Materials:

name	Revision	Modified Date
Screening questionnaire	0.01	5/23/2006 2:27 PM
UPCON Poster	0.01	1/11/2006 1:10 PM
UPCON display ad	0.01	1/11/2006 1:09 PM

66.2	* Will subjects be recruited in person for this study? no If *Yes*: Explain who will approach potential subjects to take part in this study:
66.3	* Are subjects chosen from private medical, psychiatric or academic records? no

	If *Yes*, Do any investigators on this study have routine access to the records?
66.4	* Does this study include subject chart or record review only? no

form #

67. Subject Payment/Incentives	
67.1	* Will subjects receive any payment/incentive for participation? yes If *Yes*, Describe. Include payment schedule, if applicable. Subjects will be paid an honorarium of $100 after completing the screening visit, $400 after completing visit 4, $400 after completing visit 7, and $100 for completing the entire study, for a total of $1,000. **[Include in both the protocol and the Consent form(s)].**
67.2	Specify form(s) of subject payment: no Cash yes Check no Gift certificate no Other: **If other, provide type of payment or incentives below:**

form #

77. Risks and Benefits	
77.1	Check any applicable possible risks or potential harms to subjects:
	no Use of deception yes Physical injury or discomfort yes Stress no Manipulation of psychological or social variables such as social isolation or psychological stresses yes Discovery of previously unknown condition (e.g. disease, suicidal intentions, depression, genetic predisposition): Specify condition and explain how this knowledge will be handled: Conditions such as anemia, hypertension, asthma could be discovered in the process of screening for this study. The subject will be counseled by a study physician, and referred to his/her primary care physician. yes Invasion of subjects privacy no Invasion of privacy of individuals other than the subject

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	no Risk to reputation or risk of financial harm no Social or legal risk yes Materials that may be sensitive, offensive, threatening or degrading no Other risks: If other risks, describe:
77.2	* Describe the protections that will be implemented to minimize risks or harms of **all** items checked: It is very unlikely that these exposures to concentrated outdoor particles will cause symptoms or clinically important effects. Similar studies are currently ongoing, using the same Harvard ultrafine particle concentrator, at the U.S. Environmental Protection Agency. They have nearly completed a study in healthy subjects and have found no adverse effects. Our previous studies of exposure to UFP at 50 µg/m3, with intermittent exercise, were without adverse effects. This study will be conducted at rest, which will further reduce the particle dose. In a separate study ("Inhaled particle characteristics and early lung effects," RSRB #8126), healthy subjects have been exposed to ultrafine and fine zinc oxide particles at a concentration of 500 µg/m3 without adverse effects. Previous human studies of exposure to fine carbon particles found no clinical effects of exposure to 250 µg/m3 for 1 hour or 500 µg/m3 for 2 hours. The National Ambient Air Quality Standard for outdoor particulate matter in the air (PM2.5) is 65 µg/m3, averaged over 24 hours. There is a small, theoretical risk of precipitating a cardiac event in persons with severe coronary artery disease. Therefore, subjects will have a screening ECG read by a cardiologist prior to exposure. Nevertheless, it is possible that subjects recruited for this study could have clinically silent cardiovascular disease, that might not be detected by the screening examination and ECG. We feel that the risk for precipitating a cardiac event in these exposures is extremely small, even for a subject with unrecognized coronary artery disease. First, the particles used in this study will be outdoor particles similar to what people breathe every day. The number concentrations will be higher than people usually inhale in Rochester, but will be similar to what people breathe when driving on a busy highway, or working in certain occupations. Second, all exposures will be conducted at rest, as opposed to our previous studies in healthy subjects, which have generally involved exercise. Thus the actual dose of particles to the lung will be lower in this study, and the cardiovascular stress of exercise will be absent. Finally, the risk of cardiovascular events associated with outdoor air particles is relatively small, and has required studies of millions of people to detect. While this risk is important from a public health standpoint, the net increase in risk for individuals on any given day of exposure remains very small. For example, Peters et al. found in a study of residents in the Boston area, an odds ratio for MI of approximately 1.3 associated with an increase in air pollution from the cleanest days to the worst days. The age-adjusted incidence of first acute MI for adult males is approximately 10 per 1,000 person-years, or 10 per 365,000 person-days. Thus, according to the Peters data, the risk of having a heart attack related to the worst air pollution day in Boston would increase from 1 in 36,500 to 1 in 28,077. The risk would be lower for people without clinical evidence of coronary artery disease. Given that the exposures used in this study are for only 2 hours rather than a whole day, the risk of precipitating a cardiovascular event is extremely small. The subject will be under direct observation by a trained investigator at all times during the exposure. Monitoring facilities provide continuous measurements of particle concentrations.

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	Symptoms and sensitive measurements of pulmonary function will be monitored before and after exposure. Exposures will be discontinued if any adverse symptoms occur. All subjects will remain in the Clinical Research Center for approximately 6 hours after exposure and symptoms will be assessed before discharge. Finally, subjects will return approximately 24 and 48 hours after each exposure to assess possible delayed effects. The human exposure facility is located in MRBX, a short walk from Strong Memorial Hospital. Physician availability is assured at all times.
77.3	* Describe the anticipated benefits of this research (Do not overstate): Information gained from this research will increase our understanding of the mechanisms for the health effects of air pollution particles, and aid in the determination of appropriate air quality standards.

form #

78. Data Safety Monitoring Board (DSMB)	
78.1	* Will this study use a Data Safety Monitoring Board? no
78.2	* Will an independent monitor (e.g. NCI, sponsor) audit this study? no

form #

81. Procedures and Billing	
81.1	* List the research procedures and indicate who is billed for each procedure: N/A
81.2	* List the standard of care procedures and indicate who is billed for each procedure: N/A
81.3	* List procedures that delay or preclude standard of care treatment: N/A

form #

68. Confidentiality of Data, Including Recording and Photographs			
68.1	Check types of subject identifiers that will be collected for this research. Check **all** that apply.		
	yes Name	yes Address	yes Telephone No.
	no E-mail Address	no Fax No.	yes Zip Code
	no Account No.	no Medical Record No.	no Health Plan Beneficiary No.
	no License No.	no License Plate No.	no Certificate No. [Including Device Serial No.]
	no Audiotapes	no Videotapes	
	no Subject Code	no Website URL Address or Internet IP Address	yes Social Security No.[1]
	no Finger or Voice Prints	no Full face Photographs/Images	
	no Student ID number		

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	yes Dates [Such as birth date, date of death, admission or discharge date] no None of the above [No identifiers will be collected]		
	[1]Indicate specific purpose for use of Social Security Numbers: Required for IRS reporting of payments.		
68.2	If any identifiers are checked, explain how you will protect against disclosure of these identifiers: Data will be recorded in bound laboratory books and transferred to and stored in a desktop computer using Excel software. Data will be transferred to the CRC server for preparation of SAS datasets. Only the investigators and technicians directly involved in data acquisition and analysis will have access to the data. Samples and data will be coded to protect the identity of the subjects. Computers and data books will be stored in a locked laboratory.		
68.3	* Explain how long you will keep this research data and how you will store/secure the data: Data will be kept indefinitely, under secure lock and key or password protected.		
68.4	* Will any non-study personnel (including the sponsor) have access to subject data? yes If *Yes*: Specify: University of Rochester; the Department of Health and Human Services; and the U.S. Environmental Protection Agency.		
68.5	* Does this study involve "genetic testing"? no If *Yes:* Contact RSRB office for further information. * Does this study involve keeping the data/samples for possible future research studies (e.g. tissue banking)? yes If *Yes*: Could the future research involve Genetic Testing? no		

form #

82. Federal Certificates of Confidentiality	
82.1	* Will this study include a Certificate of[1] Confidentiality? no If *Yes*, provide the expiration date of the certificate:
	*[1]Although not routinely required, if the data collected contains information about illegal behavior, genetic or other sensitive information, you may wish to visit the NIH Certificates of Confidentiality website at **http://grants1.nih.gov/grants/policy/coc/** for information on how to obtain a Federal Certificate of Confidentiality. The RSRB may require you to obtain this certificate.*

form #

83. Informed Consent Process

No Title

83.1	How will you obtain subject consent for this study?
	yes **Written Consent: Attach copy of all consent/permission/assent forms.** name Revision Modified Date UPCON consent 0.04 5/23/2006 11:24 AM
	no **Verbal Consent: Include request for waiver of documentation of consent.Attach written scripts for verbal consent/permission/assent.** name Revision Modified Date There are no items to display.
	no **Consent for Deception Study**. Attach the following: no **Consent to Procedures** and no **Consent for Use of Data** name Revision Modified Date There are no items to display.
	no **No Consent: Include a request for waiver of consent.**

* Required field

form #

85. Informed Consent Process Part II	
85.1	* Will anyone other than personnel listed on page 1 obtain consent for this study? yes If Yes, list name(s): Carol-Lynn Petronaci Alpa Shah
85.1.1	Include HSPP or EPRP numbers for all personnel who have contact with subjects and are not listed on page 1: Carol-Lynn Petronaci 36460909 9/30/07 Alpa Shah 31870808 8/31/06
85.1.2	* Will the consent form be given to subject or guardian to read prior to discussing the consent in detail? yes
85.1.3	* Will the subject or guardian be allowed to take the consent form home, if requested, to discuss with family members? yes
85.1.4	If you answered *No* to *Q85.1.2*, *Q85.1.3*, explain:

form #

70 Part 1. Use of Protected Health Information (PHI)*: HIPAA Requirements	
70.1	* Is your department/organization considered a University of Rochester "covered entity"? yes
70.2	* Is the PI or any other study personnel part of the "covered entity"? yes

form #

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70 Part 2. Use of Protected Health Information (PHI)*: HIPAA Requirements
** PHI is defined as individual health information that: (1) is created or received by a health care provider, health plan, employer or health care clearinghouse; and (2) relates to the past, present or future physical or mental health or condition of an individual; the provision of health care to an individual; or the past, present or future payment for the provision of health care to an individual.*

70.3	* Will you collect any subject PHI * as part of this study? yes If *Yes*: Indicate how you will comply with the HIPAA Requirements for this study. Check one of the following:
	no De-identification of Data[1]
	no Limited Data Set and Data Use Agreement[2]
	yes HIPAA Authorization [Include HIPAA language in consent form(s) and specify that subjects will receive a signed copy of the consent/authorization.]
	no Waiver of HIPAA Authorization [Include a request for a Waiver of HIPAA Authorization].
	[1]Refer to URMC/Strong Health Policy OP25, Appendix A. (Requires URMC Username and Password) *[2]Refer to URMC/Strong Health Policy OP25, Page 5, Item #6 (Requires URMC Username and Password)*

form #

Important: *If this application is not yet complete, leave the selection 'No' below. This will save the information you have entered to date and allow you to log back in and complete the application at a later time. If the application is complete, change the selection to 'Yes' below. Remember that completing the application does not mean it is submitted, just that you are done with entering information.*

*** Is this Application completely filled out? yes**

no Check here, if any study documents are not available electronically and deliver a **hard copy** of the document(s) to the RSRB within 1 business day.

Does your department conduct on-line review and approval of studies? yes

For Departments that Do Conduct On-line Review
When the PI presses the 'Submit Application' buttons, the completed application and attachments (uploaded files) will be forwarded electronically to your designated department reviewer.

Note: *If you have not uploaded all documents (e.g. protocol, grant, etc.) to the application, you will need to supply those in hard copy to the department reviewer and the RSRB.*

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For Departments that Do Not Conduct On-line Review

You will need to print the application (using the 'Print View' button) and send it and all hard copy documents to your department reviewer. After departmental sign-off, the PI can press the 'Submit/OK' buttons to submit the application to the RSRB. The signed department' approval (including hard copies of all documents not uploaded in the online system) must then be submitted to the RSRB.

form #

Final Instructions

You have indicated that your application is now complete. Clicking the 'Finish' button below, will take you back to your workspace. Your study will be in the '**Pre-Submission**' stage. Here you will be able to view the application in two ways. The 'View/Application Form' button takes you page by page through the application. Click the 'View/Print Application' button to view the entire application using the scroll tool or to print the application. In the '**Pre-Submission**' stage, you may also go back into the application (using the 'View/Application Form' button) to add attachments (upload documents) or modify your responses.

Submission

Once complete and ready for submission, the Principal Investigator must click the 'Submit' button. Once the application has been submitted, the application becomes 'read-only', i.e. you will not be able to access the application to make changes. Revisions or additions can only be made if the Department Reviewer or the RSRB sends the application back to you for changes.

Remember! The RSRB review process does not begin until department approval (either online or written) has been received.

form #

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UNIVERSITY OF
ROCHESTER
MEDICAL CENTER

EASTMAN DENTAL CENTER
SCHOOL OF MEDICINE AND DENTISTRY
SCHOOL OF NURSING
STRONG MEMORIAL HOSPITAL
UNIVERSITY MEDICAL FACULTY GROUP

DEPARTMENT OF MEDICINE
PULMONARY AND CRITICAL CARE UNIT

Mark J. Utell, M.D. Director

Fellowship Program Directors
Michael J. Apostolakos, M.D., Critical Care Medicine
Mark W. Frampton, M.D., Pulmonary and Critical Care Medicine

Faculty
Donald W. Greenblatt, M.D.
Richard W. Hyde, M.D.
Michael C. Kallay, M.D.
F. Eun-Hyung Lee, M.D.
Michael J. Larj, M.D.
Paul C. Levy, M.D.
Joseph E. Modrak, Jr., M.D.
Carlos R. Ortiz, M.D.
Irene B. Perillo, M.D.
Anthony P. Pietropaoli, M.D.
Patricia J. Sime, M.D.
David R. Trawick, M.D., Ph.D.
R. James White, M.D., Ph.D.

Consent Form

Study Title: Exposure to Concentrated Outdoor Ultrafine Particles in Healthy Subjects("UPCON")

Principal Investigator: Mark W. Frampton, MD

Introduction:

This consent form describes a research study and what you may expect if you decide to participate. You are encouraged to read this consent form carefully and to ask the person who presents it any further questions you may have before making your decision whether or not to participate. This study is being conducted by Dr. Mark Frampton of the Pulmonary and Critical Care Medicine Division of the Department of Medicine at the University of Rochester Medical Center.

You are being asked to participate in this study because you are a healthy nonsmoker 30 to 60 years of age.

Purpose of Study

The purpose of this research study is to determine if people exposed to very small ("ultrafine") particles normally present in the outdoor air develop temporary changes in their lungs or blood vessels. The levels of pollutants to which you will be exposed will not be higher than what you could be exposed to if you visited many major cities around the world.

Description of Study Procedures

If you agree to participate in this study, you will be asked to come to the Clinical Research Center (CRC) or the Pulmonary and Critical Care Unit on 7 separate days, including 2 overnight stays, for a total of about 66 hours over approximately 6 weeks.

On the first day (**Visit 1**), you will complete a standardized questionnaire for assessment of respiratory symptoms and medical history requiring about 15 minutes. You will have a medical and physical examination, routine breathing tests, and an electrocardiogram. A pregnancy test will be performed in female subjects. The pregnancy test must be negative. Visit 1 takes approximately 2 hours and will determine whether you are eligible to participate in this study.

You will be required to avoid the following medications during and for the indicated interval before the study. You must be able to avoid the medications listed in this table for the time indicated at the top of each column:

ONE MONTH	ONE WEEK	ONE DAY TO ONE WEEK
Systemic steroids such as prednisone	Non-steroidal anti-inflammatory drugs such as ibuprofen, naproxen, including aspirin Vitamins C and E Antihistamines Anti-oxidants Fish oil Niacin Arginine Over-the-counter decongestants	Sildenafil (Viagra) (36 hours) Vardenafil (Levitra) (36 hours) Tadalafil (Cialis) (87 hours)

You will also be asked to follow a low nitrate and non-caffeine diet on all study days, including the two days after each exposure. You will be given a list of both appropriate foods and those to be avoided.

One or more days after Visit 1, you will be asked to come to the CRC at noon (**Visit 2**), having eaten a low-nitrate breakfast at home. You will be rescheduled if you have experienced an upper or lower respiratory tract illness within the past 6 weeks, or any other acute illness within the past week. Women will be asked about their latest menstruation and a urine pregnancy test will be conducted. If you are pregnant your participation in the study will end and you will receive full compensation for the exposure day. You will be provided with lunch. At about 1 PM you will have the following procedures: blood pressure, heart rate, attachment of a finger clip and recorder for the measurement of oxygen in the blood (oximetry), completion of a questionnaire about symptoms, a forearm blood flow test by brachial artery ultrasound, measurement of lung diffusion (breathing tests to measure how well oxygen and other gases get into the lung), measurement of the amount of nitric oxide in your exhaled breath, removal of blood (about 3 tablespoons) from a vein in your arm, and routine breathing tests. You will be given a recording device (Holter monitor) and adhesive patches will be placed on your chest to record your heartbeat, with a resting recording of 10 minutes. These procedures will take about 2 hours. You will be given dinner that evening, and will stay on the CRC overnight.

The next morning you will have a light breakfast at 6:30 AM. At 7:30 AM you will be transported by wheelchair to the Kornberg Medical Research Building, where you will have a 2-hour exposure to either clean air or clean air containing concentrated outdoor ultrafine particles. You will not be told which exposure you are receiving, and the investigators will not know. Only the person operating the

exposure equipment will know which exposure is being given. The order of giving air or particles will be chosen at random (like flipping a coin).

The exposure will be done inside a Plexiglas chamber (6 x 5 x 3.5 feet, 98 cubic feet) in a research building at the Medical Center. The chamber will be under negative air pressure, which may make your ears pop, like going down in an elevator. On the particle exposure day, the air you breathe will contain particles from outside the building that have been concentrated about 10 to 20 times more than their concentration outdoors. The amount of particles you will be exposed to will depend on the amount of pollution in the outside air on the day of your exposure. A trained investigator will be nearby to observe you at all times. A physician will be on call in the facility during the entire exposure session.

It is not expected that the exposures in this study will cause any symptoms. If it appears you are experiencing any problems, or you develop any symptoms of discomfort, the exposure will be stopped immediately. In addition, you may choose to stop the exposure at any time for any reason. If you do so, you will be paid in full for that day's session, but will be ineligible for further participation in the study and for any further payments.

After the exposure, blood pressure, heart rate, and blood oxygen saturation will be measured, and you will be given a questionnaire to record symptoms. Then you will be transported back to the CRC. You will be given lunch at noon. At 1 PM the following measurements will be made: blood pressure, heart rate, oximetry, a symptom questionnaire, 10-minute heart recording, forearm blood flow test, blood tests, and lung function tests. All of these measurements are described in detail below. You will then go home with a heart monitor and be asked to record your activities in a diary. The total time for Visit 2 will be about 27 hours, including the overnight stay.

You will return the next morning (**Visit 3**), approximately 24 hours after exposure. Before coming in for Visit 3, you will be allowed to remove the heart monitoring electrodes to shower or bathe. Upon arriving for Visit 3, monitoring electrodes will be re-applied to your skin and an oximeter will be placed on your finger. All of the measurements listed above will be repeated. You will be asked to hand in your activity diary. You will go home with another activity diary and the heart monitor in place for another 24 hours. Visit 3 will take approximately 3 hours.

You will return again the following morning (**Visit 4**), about 48 hours after exposure, for the same tests as in Visit 3. The heart monitor will be removed at this time, and you will hand in your activity diary. Visit 4 will take approximately 3 hours.

At least 3 weeks after Visit 2, you will return for **Visits 5, 6, and 7**. The procedures performed on Visits 2, 3, and 4 will be repeated. You will have completed the study after Visit 7.

The measurement procedures are described below:

1) Routine breathing tests (spirometry and lung volumes). These tests require you to perform 3 to 5 forceful exhalations after a deep breath (spirometry) and/or breathe inside a chamber (like a telephone booth) to measure the volume of all the air in your lungs. This test is performed routinely on patients and does not carry significant risks.

2) Blood drawing. Blood will be removed from a vein in your arm for the study of blood cells and fluids. The amount of blood taken at each blood drawing will be approximately 3 tablespoons at a time and no more than 250 ml over the three visits of an exposure session. In this study, you will have the option of agreeing to have your blood sample stored for future research. If you agree, you may be contacted in the future to obtain consent for genetic testing.

3) Measurement of lung diffusion. These are two tests that measure how quickly certain gases in the air get into the lung. The first test measures the diffusing capacity for carbon monoxide, or DLCO. This is part of routine breathing tests and involves inhaling air with a tiny amount of carbon monoxide and helium, holding your breath for 10 seconds, and then breathing out. The second test measures the diffusing capacity for nitric oxide, or DLNO. Nitric oxide is a gas that is produced and released in very small quantities by many cells in the body. You will inhale a low concentration (up to 10 parts per million) of nitric oxide from a bag and then hold your breath for 3 to 5 seconds followed by a slow exhalation. This will be repeated 3 times. This concentration of nitric oxide is higher than that found in fresh air, but is below the levels that have been measured in air that is normally found in the stomach or in the sinuses of the nose. The concentration is also below that which is commonly used for medical treatment of various lung diseases. There are no known undesirable effects from either of these tests.

4) Measurement of nitric oxide in the exhaled air. Nitric oxide is a gas that is produced and released in very small quantities by many cells in the body. It is released by cells of the respiratory tract, and can be measured in air exhaled from the lungs. People who have irritation or inflammation in the lungs sometimes have increased amounts of nitric oxide in their exhaled air. We will determine whether exposure to ultrafine particles causes exhaled nitric oxide levels to increase. You will be asked to perform a series of breathing maneuvers through a mouthpiece. You will be asked to hold your breath for 10 seconds and to breathe out against a slight resistance. This will be done 6 times, each time breathing out at different speeds. During these tests, you will be inhaling air that has been purified to remove nitric oxide.

5) Heart monitoring (Holter monitoring). This involves the placement of leads or electrodes using adhesive on your body to monitor the heart's electrical activity. Chest hair may need to be shaved before placing the electrodes. The leads are connected to a recording device, which is carried on a belt. You will wear the heart monitor during the exposure and at home overnight for a total of 2 days, and keep a diary of your activities during that time. There is no significant risk associated with heart monitoring, although there may be itching or even a rash where the leads are placed on the chest. You will not be able to shower or swim while the monitor is in place, but on the mornings of Visits 3 and 6 you can remove the monitor to shower or bathe before coming to the clinic.

6) Forearm blood flow test. This test uses ultrasound to measure changes in the size of the blood vessel in your arm. We will also be able to calculate the flow of blood through that vessel. It involves a blood pressure cuff to be applied to your lower arm and restricting circulation for five minutes by inflating the blood pressure cuff. The size of the artery in your arm is then measured by ultrasound. After these measurements you will rest quietly for 10 minutes. You will then be given a nitroglycerine tablet under the tongue, which dilates the blood vessels. 3 to 4 minutes later the forearm blood flow test will be repeated. Forearm blood flow testing is a well-recognized, non-invasive measurement of the ability of

blood vessels to enlarge following a brief period of lack of blood flow. It takes about 30 minutes. Subjects may experience tingling of the hand or even brief numbness while the blood pressure cuff is inflated. Occasionally, nitroglycerine can cause a brief headache or lightheadedness. You should not be taking any drugs for erectile dysfunction, like Viagra, Levitra or Cialis, because taking nitroglycerine can cause low blood pressure if you are taking these drugs.

The Table below shows when these procedures will be performed.

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
History and Physical Examination	X						
Blood Pressure and Heart Rate	X	X	X	X	X	X	X
Oximetry	X	X	X	X	X	X	X
Electrocardiogram	X						
Heart Monitoring		X	X	X	X	X	X
Blood Drawing	X	X	X	X	X	X	X
Pregnancy Test	X	X			X		
Symptom Questionnaire		X	X	X	X	X	X
Blood Flow Test		X	X	X	X	X	X
Air/Particle Exposure		X			X		
Routine Breathing Tests	X	X	X	X	X	X	X
Lung Diffusion		X	X	X	X	X	X
Measurement of Exhaled Nitric Oxide		X	X	X	X	X	X

Number of Subjects

Twenty subjects are needed to complete this study.

Risks of Participation

The particles you breathe will come from outdoor air pollution that we all breathe. The number of particles will be higher than normally occur outdoors in Rochester.

Possible effects of exposure to ultrafine particles are cough, shortness of breath, or irritation of the throat. However, these low concentrations of particles are not expected to cause any symptoms. Large epidemiology studies indicate that exposure to air pollution particles may increase the risk of having a heart attack or other heart problems in people who have heart disease. If you have significant symptoms such as chest pain or shortness of breath, the exposure will be stopped.

Breathing tests (spirometry) may induce lightheadedness as a result of taking a deep breath 3 times in a row.

Blood drawing may cause pain and bruising at the place where the blood is taken. Rare complications from blood drawing include, but are not limited to, blood clots, infection, and light-headedness or fainting.

Measuring the exhaled nitric oxide may cause slight discomfort and drying of the nose or mouth.

Forearm blood flow test may cause temporary numbness of the hand and arm. Taking nitroglycerine can cause headache, a drop in your blood pressure or lightheadedness.

Questions on the health questionnaire will ask about smoking and drinking habits, which may make you feel uncomfortable. You are free to choose not to answer any question for any reason.

Benefits of Participation

There are no benefits that you can expect to receive as a result of participating in this study.

Alternatives to Participation

You may choose not to participate in this study.

Payments

You will be paid $100 after completing Visit 1, $400 after completing Visit 4, $400 after completing Visit 7, and $100 for completing the whole study, for a total of $1,000.

Sponsor Support

The University of Rochester is receiving payment from the Department of Health and Human Services and the U.S. Environmental Protection Agency for conducting this research study.

Confidentiality of Records and HIPAA Authorization

While we will make every effort to keep information we learn about you private, this cannot be guaranteed. Other people may need to see the information. While they normally protect the privacy of the information, they may not be required to do so by law. Results of the research may be presented at meetings or in publications, but your name will not be used.

The Federal Health Insurance Portability and Accountability Act (HIPAA) requires us to get your permission to use health information about you that we either create or use as part of the research. This permission is called an Authorization. We will use your health information to conduct the study, to monitor your health status, to measure the effects of inhalation of particles, and to determine research results. Health information is used to report results of research to sponsors and federal regulators. It may be audited to make sure we are following regulations, policies, and study plans. Strong Health policies let you see and copy health information after the study ends, but not until the study is completed. If you have never received a copy of the Strong Health HIPAA Notice, please ask the investigator for one.

To meet regulations or for reasons related to this research, the study investigator may share a copy of this consent form and records that identify you with the following people: the University of Rochester; the

Department of Health and Human Services; National Institute of Environmental Health Sciences and the U.S. Environmental Protection Agency.

If you decide to take part, your Authorization for this study will not expire unless you cancel (revoke) it. The information collected during your participation will be kept indefinitely. You can always cancel this Authorization by writing to the study investigator. If you cancel your Authorization, you will also be removed from the study. However, standard medical care and any other benefits to which you are otherwise entitled will not be affected. Canceling your Authorization only affects uses and sharing of information after the study investigator gets your written request. Information gathered before then may need to be used and given to others.

As stated in the section on Voluntary Participation below, you can also refuse to sign this consent/Authorization and not be part of the study. You can also tell us you want to leave the study at any time without canceling the Authorization. By signing this consent form, you give us permission to use and/or share your health information as stated above.

Contact Persons

For more information concerning this research or if you believe that you have suffered from a research-related injury, you should contact Dr. Mark Frampton at (585) 275-4161.

If you have any questions about your rights as a research subject, you may contact the Human Subjects Protection Specialist at the University of Rochester Research Subjects Review Board, Box 315, 601 Elmwood Avenue, Rochester, NY 14642-8315, telephone (585) 276-0005; for long-distance you may call toll-free, (877) 449-4441.

Voluntary Participation

Participation in this study is voluntary. You are free not to participate or to withdraw at any time, for whatever reason, without risking loss of present or future care you would otherwise expect to receive. In the event that you do withdraw from this study, the information you have already provided will be kept in a confidential manner.

Signature/Dates

Study Subject:
I have read the contents of this consent form, and have been encouraged to ask questions. I have received answers to my questions. I agree to participate in this study. I have received (or will receive) a signed copy of this form for my records and future reference.

I agree to have my blood samples used for other non-genetic research in the future. Samples will be identified only with my initials and a number, not my name.

I do not agree to have my blood samples stored for other non-genetic research in the future.

_____**PRINT NAME**

_____**SIGNATURE** _____**DATE**

Person Obtaining Consent:

I have read this form to the subject and/or the subject has read this form. The subject will receive a signed copy of this consent form. An explanation of the research was given and questions from the subject were solicited and answered to the subject's satisfaction. In my judgment, the subject has demonstrated comprehension of the information.

_____**PRINT NAME AND TITLE**

_____**SIGNATURE** _____**DATE**

PROTOCOL SUMMARY

Exposure to Concentrated Outdoor Ultrafine Particles in Healthy Subjects ("UPCON")

Exposure (Visits 2 and 5)

Time	Activity
Day prior to exposure:	
12:00	Subject arrives, given lunch on CRC
13:00	Exam and vital signs (BP, HR)
	Oximetry
	Symptom questionnaire
	Holter monitor attached
	Holter recording
	Forearm blood flow
	Phlebotomy
	DLCO
	DLNO and Exhaled NO
	Spirometry
18:00	Dinner, stay overnight
Day of exposure:	
07:00	To exposure lab
07:30	Exposure begins (air or UFP)
09:30	Exposure completed, return to CRC
12:00	Lunch on CRC
13:00	Vital signs and oximetry
	Symptom questionnaire
	Holter monitor attached
	Holter recording
	Forearm blood flow
	Phlebotomy
	DLCO
	DLNO and Exhaled NO
	Spirometry
15:30	Leave CRC with Holter and diary

1st Post exposure Day (Visits 3 and 6)

Time	Activity
08:00	Subject arrives at CRC
08:15	Vital signs and oximetry
	Symptom questionnaire
	Hand in diary
	Holter recording
	Forearm blood flow
	Phlebotomy
	DLCO
	DLNO and Exhaled NO
	Spirometry
11:00	Leave CRC

2nd Post exposure Day (Visits 4 and 7)

Time	Activity
08:00	Subject arrives at CRC
08:15	Vital signs and oximetry
	Symptom questionnaire
	Hand in diary
	Holter recording
	Forearm blood flow
	Phlebotomy
	DLCO
	DLNO and Exhaled NO
	Spirometry
11:00	Leave CRC